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A NOTE ON WOUNDING OF THE LEAVES OF *ANACARDIUM OCCIDENTALE* LINN. AT DIFFERENT STAGES OF THEIR DEVELOPMENT AND ITS EFFECT ON RESPIRATION. *

BY

A. B. SARAN

Variations in the initial rate of respiration exhibited by young growing organs of different species of plants, have been fully investigated by Bezagu (3), Bonnier and Mangin (4), Borodin (5), Corenwinder (7), Nicolas (12) and others. That wounding enhances respiration has also been investigated by several workers, chief amongst whom are Palladin (13), Boehm (6), Lutman (10), Magness (11), Stich (15), Johnston (9), Richard (14), Audus (1), Godwin (8), and Barker (2). There seems, however, so far as known no record of work done elucidating the phenomenon of respiration on wounding of plant organs in different stages of their growth. Experiments were, therefore, conducted to investigate this aspect of the problem with the leaves of *Anacardium occidentale* Linn.

Material and method: Leaves for the experiment were invariably taken from a particular branch of a fully grown tree. Age of the leaf was calculated from the date it fully unfolded itself out of the bud. The leaves on isolation were weighed and kept with their petioles dipping in water in

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* This work was undertaken and completed at the Botanical Laboratory, Ravenshaw College, Cuttak, during the year 1932.

Table I showing the rate of respiration before and after wounding the leaves in various stages of growth.

Age of leaf in days.	EXPERIMENTAL			CONTROL		
	Output of CO$_2$ in mgs. per 10 gms. of fresh leaf per two hours.		After-effect i.e. difference between the output of CO$_2$ before and after wounding.	Output of CO$_2$ in mgs. per 10 gms. of fresh leaf, per two hours, corresponding to the periods A & B, respectively of the previous column.		Difference between the first and second two-hourly readings.
	A	B		First two-hourly readings.	Second two-hourly readings.	
	Just before wounding.	Just after wounding.				
3	26.5	18.4	—8.1	26.1	18.2	—7.9
8	17.0	12.4	—4.6	16.3	12.2	—4.1
14	14.0	10.1	—3.9	14.3	11.0	—3.3
20	13.7	10.5	—3.2	13.4	10.4	—3.0
30	9.9	11.8	+1.9	9.8	8.0	—1.8
40	10.1	11.8	+1.7	8.9	7.1	—1.8
100	9.4	11.2	+1.8	9.6	8.0	—1.6

the respiration-chamber. The estimation of carbon dioxide was done by the continuous current method. The apparatus was allowed to run for half an hour before the first two hourly readings were taken. Immediately afterwards, the leaves (in the chamber) were wounded, each very approximately to the same degree along the veins and the next two hourly readings were again recorded.

Results and their consideration: The results obtained (vide Table I) are presented under each sub-head in brief.

Young Leaf: An inspection of the table will show that younger leaves display a fairly high rate of respiration as compared to old ones. The youngest in the series (*i.e.* 3 days old) exhibits a rate which is as high as 26.5 mgs. This, however, falls down to 13.7 mgs. during a period of 20 days. On wounding such young leaves (*i. e.* 3, 8, 14 and 20 days) no rise in respiration is evident. This probably is due to the fact that irritability in the leaves is not so well developed between the ages of 3-20 days.

Old leaf: The higher initial rate of respiration shown by younger leaves, finally settles down to 9-10 mgs. in the course of 30-40 days, after which even up to the 100th day, the results obtained, yield a more or less constant value (vide Table 1). An after-effect (*i.e.* a rise in respiration over the initial output of CO_2 following wounding) is first seen where 30 days old leaves are involved. The first two-hourly readings on wounding, display an enhanced rate of respiration, which is without exception followed by falling values. Similar results are obtained by other sets of older leaves (*i.e.* 40 and 100 days) as well. The values of the after-effect vary from 1.8-1.9 mgs. per two hours per 10 gms. of fresh leaves. The after-effect is, however, in every case a short lived one passing off in about 2-4 hours.

Summary

In *Anacardium occidentale* Linn. young leaves have definitely been found to respire at a higher rate than adult ones. This rate finally settles down to a lower level value in the course of 30-40 days. Such young leaves, when wounded do not show any after-effect, while a similar treatment to the older leaves enhances their respiratory activity considerably.

Literature Cited

1. AUDUS, L. J. (1935).—Mechanical stimulation and respiration rate in the Cherry Laurel. *New Phytologist.*

2. BARKER, J. (1935).—A note on the effect of handling on the respiration of potatoes. *New Phytologist.*

3. BEZAGU, (1919).—*Compt. Rend. Act. Sci.* Paris. CLXIX.

4. BONNIER, G. et MANGIN, L. (1885).—*Ann. des Sci. Nat. Bot.* Serie VII.

5. BORODIN, J. (1876).—Physiologische Untersuchungen über die Atmung der beblättern Sprosse. *Arb. der St. Petersburger Gesellsch. der Naturf.* Bd. VII.

6. BOEHM, J. (1897).—Ueber die Respiration der Kartoffel *Bot. Zeitung.*

7. CORENWINDER, (1878).—*Ann. de Chimie et Phys.* 5e Série t. XIV.

8. GODWIN, H. (1935).—The effect of handling on respiration of cherry laurel leaves. *New Phytologist.*

9. JOHNSTONE, G. R. (1925).—Effect of wounding on respiration and exchange of gases. *Bot. Gaz.*

10. LUTMAN, B. F. (1926).—Respiration of potato tubers after injury. *Bull. Torrey. Bot. Club.*

11. MAGNESS, J. R. (1920).—Composition of gases in the inter-cellular spaces of apples and potato. *Bot. Gaz.*

12. NICOLAS, G. (1918).—*Rev. Gen. Bot.* XXX.

13. PALLADIN'S PLANT PHYSIOLOGY, Eng. Trans. Edited by B. E. Livingston.

14. RICHARD, H. M. (1896).—The respiration of wounded plants. *Ann. Bot.*

15. STICH, C. (1891).—Die Athmung der Pflanzen be verminderter Sauerstoffspannung und bei verfetzungen. *Flora.*

THE EFFECTS OF RADIATION ON SOME *POLYPORES* IN CULTURE

BY

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(Presidential address delivered at the seventeenth Annual Meeting of the Indian Botanical Society at Calcutta, on 6th January, 1938.)

Before I pass on to the subject of my address, I like to place before you a short history of our Indian Botanical Society especially for the information of foreign Botanists gracing this occasion. The Indian Botanical Society was started on December 6, 1920, after deliberations amongst the members of the Botany section of the Indian Science Congress at its Nagpur sitting in consultation with Botanists from different parts of India. The late Dr. Winfield Dudgeon of Allahabad (Jamuna Mission College) took a very prominent part in its inauguration and was its first President in 1921, and the late Prof. S. R. Kashyap of Lahore (Punjab University) was its first Secretary and Treasurer for 1921-1922. The Journal of Indian Botany was started in September, 1919, for publishing botanical work done in India; the Journal owes its inception in the first place to the enthusiasm of the late Mr. L. J. Sedgwick, I.C.S., F.L.S., a keen and prolific worker on Indian systematic botany, and in the second to the kind services of Mr. T. R. D. Bell, c.i.e., the late Chief Conservator of Forests, Bombay, who generously came forward with an offer to guarantee the expenses till the Journal should be so far established as to pay its way; Prof. P. F. Fyson, B.A., F.L.S. the late Professor of Botany of the Presidency College, Madras, was its first Honorary Editor. The Journal was launched with a mixture of hope and doubt; though supported by nearly every important Botanist in India the proposal met with misgivings from several who thought ' the times were yet not ripe '. But within a very short time, in fact in the course of eight months, thanks to the active cooperation of Botanists in India, ten numbers appeared with an average of over thirty pages of original matter and diagrams and about four of abstracts (reviews) to each. The original papers have been on nearly every branch of pure Botany, *i.e.* on Fungi, Algae, Liverworts, Mosses, Gymnosperms, the Taxonomy of flowering plants,

General and Physiological Histology and Morphology, Phys-
iology, Ecology, and a Systematic Flora of a province. Abs-
tracts and reviews have appeared of over 50 papers and books,.
and occupied 40 pages of small type.

In February 1922, the Indian Botanical Society decided to·
approach Prof. P. F. Fyson to find out whether he would be·
willing to turn the Journal over to the Society. After some
correspondence, Prof. Fyson finally agreed to hand the Journal.
over to the Indian Botanical Society. The .transfer of the
Journal to the Society was finally completed in October 1922 and:
since then the Journal is the official organ of the Society. It was.
decided to change the name of the Journal from " The Journal.
of Indian Botany " to " The Journal of the Indian Botanical:
Society " in January 1923. No. 6 of Volume III of the Journal.
was the first number issued by the Society in April 1923. The·
Journal records the activities of Botanists working in India on
various phases of Botany, cytology-papers usually prepondera-
ting in number. A booklet on " The Flora of the Indus Delta "·
by Mr. T. S. Sabnis has been issued by the Society out of the·
reprints in earlier issues of our Journal. With the transfer·
of the Journal to the Indian Botanical Society an Editorial.
Board with an Editor-in-Chief was constituted for conduc-
ting the Journal. Prof. Fyson was elected the first Chief·
Editor of the Journal. He continued as Editor up to 1926, then·
it passed through the editor-ship of Prof. B. Sahni, Prof.
M. O. P. Iyengar and late Prof. S. R. Kashyap in succession..
The present Editor is Prof. P. Parija of Ravenshaw College,.
Cuttack; during his able editorship the Journal has become·
bimonthly (of about 350 pages a year), while formerly it used:
to appear quarterly. The total number of members (ordinary·
and associate) is about 150; the Business-Manager's report gives
a detailed account on these points. I am glad to record the·
good services rendered by Dr. Miss E. K. Janaki Ammal, our.
present Secretary, to the cause of the Society.

Introduction

Most of the published works on radiation in fungi are of a
decidedly qualitative nature without adequate control of environ-
mental conditions, hence it becomes difficult to arrive at a correct
interpretation of results which cannot be easily duplicated. The·
radiation here refers to treatment of malt-extract agar-plate-cultures·
of three Polypores *(Polyporus ostreiformis, Polystictus leoninus·*
and *Trametes cingulata)* by ultra-violet rays, X-rays, radium, and:
sunlight. The effects of radiation, as pointed out by Catcheside (5)
are usually manifested in two ways, either (1) by producing·
temporary physiological effects on the individual, *i.e.,* by depressing·
or accelerating physiological functions or (2) by *permanent* effects
leading to death or some modification of the germ-plasm. The
first is phenotypic and indirect, producing noxious physical or·
chemical conditions in the environment of the germ-plasm, and the·

second is genotypic, causing direct hits on the chromosomes in the nucleus, a single hit being defined as the absorption of one quantum of energy in the sensitive region. The sequence of events in chemical effects according to G. Failla (10) are (*a*) ionization (*b*) chemical changes (*c*) biological changes; this probably accounts for the delay in the appearance of the effects of radiation in living organisms. —known as the latent period.

Most of the effects on fungi are of temporary nature. Salt-ations or mutations in fungi due to the influence of radiation pro-ducing heritable changes are rather few; in the Polypores treated I could not find any. This supports my previous experience in the course of sexuality-study of Polypores (2) that additions of minute doses of poisons and acids or the variations of temperature and of light and darkness and the change of various kinds of nutritive media could not produce any mutation or saltation of the monosporous mycelium, they seemed absolutely stable, not alterable by any change of circumstances or conditions. In this connexion attention may be drawn to the relevant remarks of Burkholder (4) that "in the normal course of events, where light exerts an action upon growth, it is probably brought out by absorbing substances (pigments) normally present in the plant." The three *Polypores* treated here are perfectly white, devoid of any pigmented sub-stance. Even in the case of green plants several investigators have found that different species as well as different individuals of the same species vary in their reaction to the rays; this relates to the question of special sensitivity in some plants. Only those plants whose genes are in an unbalanced state can be easily changed by the action of X-rays, ultra-violet, etc. Radiation-experiments which I have carried on *Polypore*-cultures are summarised below under appropriate headings.

Ultra-violet radiation and solar radiation

Materials and methods

Malt-agar (malt extract 3% and agar 2%) plate-cultures of *Polystictus leoninus, Polyporus ostreiformis* and *Trametes cingulata* were used throughout. The plates were exposed to the full range of quartz mercury arc screened by cellophane paper from a *Hanovia Alpine Sun* operating on D. C. at 3.5 amperes with 100 volts across the arc. The distance of the arc from the culture-plate was in every case kept at 30 c.m., at this distance practically no heating was produced near the culture-plate. The lid of the petri dish was always replaced by a thin cellophane paper (·025 mm. thick) previously sterilised by alcohol, this prevented dust and contamina-tion while allowing U.V. rays to pass through it. The range of wave lengths was from 2400 to 4000 A°u which includes the whole of the biotic and a portion of the abiotic range and some portion of the violet region of the visible spectrum. Two kinds of exposures were given in each case—(1) an almost daily exposure of five minutes' duration for 15 days, (2) and only twice, of fifteen minutes' exposure, in a fortnight.

Results (I) in the case of daily exposure of five minutes' duration for 15 days.

(1) Damage in the main plate of *Polystictus leoninus*

In the case of *Polystictus leoninus* the plate was exposed on 9th October, 1936, just four days after inoculation of the plate so that sufficient space was left in the plate for the growth of the culture following ultra-violet radiation; in this state the vegetative hyphae were all filled up with protoplasm, they were with clamps and with a number of short mediate branches without any trace of basidia.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae The change produced after the very first exposure was considerable damage to the vegetative hyphae. Many become clampless, and protoplasm became broken up into pieces and much vacuolated. These hyphae ultimately lost their protoplasmic contents, became double-walled and were converted into hairs. Apart from mediate branches hyphae without clamps and branched at right angles were also produced in good numbers, but the number of clamped hyphae always preponderated over non-clamped ones. With increasing exposures the non-clamped hyphae were also damaged and showed broken nature of protoplasm. (*b*) Conidia were never produced. (*c*) Chlamydospores were produced in good numbers in cultures one month and ten days old from the date of inoculation.	Basidia (the evidence of fruit-formation) appeared after the third exposure i.e. seven days after the inoculation. The fruiting period in the control plate was also seven days ; thus, the initiation of the fruit-formation was neither hastened nor retarded due to radiation. But no porous surface was formed in the irradiated plate nor any regular and erect fruits were produced, as are found in the normal cultures. Smears from the coloured (yellowish) area showed the accumulation of black spots under the microscope within which were found numerous basidia and crystals of calcium oxalate. The basidia were with sterigmata and attached spores, they were irregularly scattered and never arranged in clusters ; with increasing exposures the basidia became very much shrunken in appearance.	The upper surface of the irradiated culture gradually developed a dull yellow colour after the fifth exposure which deepened with increased radiation. The yellow colour first developed in small patches scattered over the surface of the culture and ultimately the whole surface assumed a dull yellow colour, which however was not carried over to its sub-cultures and presented a sort of burnt-up appearance. The hyphae forming the aerial part of the culture plate never tended to go down and sink to the bottom of the medium to avoid strong radiation as has been reported by Weston and Halna ( ). In the course of day-to-day radiation if there was an interruption for two or three days the culture in the main plate showed a slight tendency to reversion to the normal condition.

(2) Damage in the main plate of *Trametes cingulata*

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae Slight damage to hyphae was noticed after the second exposure on 10th. November, 1936; the damage became more pronounced with increasing exposures, the hyphae lost their turgid condition and became gradually narrower than the normal ones with their protoplasm either disintegrated or broken up into disconnected masses. Empty hyphae and extremely narrow hyphae were also produced in good numbers and many of them lost their clamps. (*b*) Conidia With increasing exposures the number of conidia decreased till after the fifth exposures on 16th November, 1936 (i.e. 13 days after inoculation) they totally disappeared from the culture. (*c*) Chlamydospores were present in small numbers when exposure was first begun. Their number increased to a maxium up to the seventh exposure (on 23-11-36) after which they gradually diminished but never disappeared altogether ; a good many of them were found in empty condition.	This strain of *Trametes cingulata* was highly conidial and non-fruiting, no sporophore appeared even in normal cultures.	No Colouration of the upper surface of the culture plate was noticed as in the case *Polys. leoninus.*

(3) Damage in the main plate of *Polyporus ostreiformis*

In the case of *Polyporus ostreiformis* the strain was non-conidial with a good number of chlamydospores. A smear from the normal culture showed hyphae measuring 4—6μ in breadth with

well-developed clamps and mediate branches in good numbers. During the whole period of the experiment the normal strain was found to fruit only in solid cultures in plates and better fruiting occurred in liquid (malt-ext.) cultures in flasks. No fruiting occurred, however, in tube-cultures. Fruiting occurred in plate cultures in 19–20 days and was confined only on the walls of the plates. In liquid cultures in flasks fruiting was not only earlier but also far bigger in size than in plates. Various methods were tried to induce fruiting in tubes without any success upto January, 1937, but since February, 1937, several tube-cultures which were kept under identical conditions, fruited almost simultaneously, and since then fruiting has always been recorded in tubes.

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. No damage was noticed until after the third exposure on 11th. November 1936. Even then the damage was slight and in smears the hyphae were hardly distinguishable from the normal ones. Damage became more pronounced after the seventh and eight exposures and went on increasing with increased doses of radiation. The nature of damage noticed was the same as in other cases. The protoplasm became more and more vocuolated and broken up into disconnected chains with a strong tendency to break up into oidia. Several hyphae lost their protoplasmic contents altogether and a large number of empty hyphae could be found without any clamps. Those that were living became narrower till at the end they became converted into a large number of extremely narrow hyphae containing disconnected masses of protoplasm.	No fruiting ever occurred the irradiated plate but a smear taken 27 days after inoculation from condensed area on the wall of the plate showed the presence of a good number of rudimentary basidia without any sterigmata or spores. No porous area, however, was formed.	Resistence to damage seemed to be more marked in P. leoninus or T. cingulata and whereas in the latter damage was evident either after the first or second five minutes exposure in P. ostreiformis no damage was noticed until after the third exposure. The only noticeable point was the development of a good number of uniformly narrow filled up and elongated hyphae without clamps showing no trace of damage But considering the number of extremely damaged narrow hyphae which still retained clamps at every partition, the number of these narrow healthy hyphae without clamps was not at all greater. Hyphae with clamps always preponderated over those without clamps whether they were filled up or damaged. The number of mediate branches neither increased nor decreased and they were damaged to the same extent as the main hyphae.

In the vegetative stage.	In the reproductive stage.	Observations.
(*b*) Conidia. A few conidia were developed after the thirteenth exposure on 7th December, 1936 (34 days after inoculation) but their number never increased. (*c*) Chlamydospores were in fairly good number in the beginning. Their number gradually went on increasing but after nine exposures they rapidly diminished in number but never vanished altogether.		

(1) Changes in sub-cultures from the main plate of *Polystictus leoninus* (*i.e.*, in the first vegetative generation.)

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In *Polystictus leoninus* altogether five subcultures were carried out in malt-agar plates from the irradiated plate, the first after the first exposure, the second after the third exposure, the third after the ninth exposure, the fourth after the fourteenth exposure, the fifth and the last ten days after the fifteenth exposure. Smears from the vegetative area showed hyphae with much broken up protoplasm and much narrower than the normal ones with clamps extremely small and reduced in size. Many of these were ultimately converted into hairs. Hyphae devoid of	The first and the second subcultures fruited after 8 days while the last 3 subcultures fruited on the 9th day after inoculation. The fruiting period in the control at that time was 6 to 7 days so that fruiting (as noted by the 1st appearance of basidia in smears) was delayed by 1 to 3 days. Toothed area developed all round the inoculum and smears from these areas showed the presence of numerous basidia arranged in close clusters but very few were with attached spores. The erect fruits that were developed later in these subcultures were found on sectioning to have empty pores in the majority of cases with a few tramal hyphae	

In the vegetative stage.	In the reproductive stage.	Observations.
clamps were produced in good numbers, but hyphae with clamps always preponderated. (*b*) The number of conidia produced was small but later on they disappeared altogether. (*c*) Another feature of these 1st. generation sub-cultures was that chlamydospores were produced in large numbers from the beginning—a condition not found in the normal.	bearing clamps only at places. These fruits on being inserted in agar plates did not shed any spores. The shapes of these fruits were irregular when fully formed.	It is thus seen that the damage due to ultraviolet rays persisted right through the first generation (in plate) and no tendency to reversion was noticed either in the reproductive or in the vegetative phase.

(2) Changes in sub-cultures from the main plate of *Trametes cingulata* (*i.e.*, in the first vegetative generation)

In the vegetative stage.	In the reproductive. stage.	Observations.
(*a*) Hyphae. In the case of *Trametes cingulata* altogether 4 subcultures were carried out from the main plate after the third, fifth, twelfth and fifteenth exposures. The first two subcultures showed slight damage in the beginning but these totally disappeared later, while the last 2 subcultures showed complete reversion to the normal state. Recovery, therefore, occurred in the 1st. generation subcultures. No transfers were made to woodblocks. (*b*) Conidia appeared in large numbers. (*c*) A good number of chlamydospores were present.	No fruiting occurred anywhere even in controls, the strain being non-fruiting.	Damage was first noted on the 11th. Nov. 1936, and complete recovery was noted in the 1st. subculture on the 2nd. Dec. 1936, within an interval of 22 days. Subcultures were carried upto third generation but in no case the damage was found to persist.

(3) Changes in sub-cultures from the main plate of *Polyporus ostrieformis.* (*i.e.,* in the first vegetative generation.)

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In the case of *Polyporus ostrieformis* altogether 4 subcultures were carried from the irradiated plate. The 1st was after the third exposure (just after the damage was noted) on the 11th Nov. 1936; the 2nd after the fifth exposure on the 15th Nov. 1936; the 3rd after the 12th exposure on the 5th Dec. 1936; the 4th and the last after the 15th exposure on the 11th Dec. 1936 (in tube). Smear examination showed that the damage caused by irradiation in the main plate was partly maintained in the 1st generation sub-cultures. Empty hyphae together with a large number of narrow hyphae retaining extremely narrow clamps and with protoplasm broken up into disconnected masses were found mixed with a large number of healthy hyphae with normal clamps and mediate branches, so that unlike *T. cingulata* recovery in the 1st generation was only partial. A fair number of narrow filled up and elongated hyphae without clamps were found as in the irradiated main plate. (*b*) Conidia developed in small numbers as in the irradiated plate. (*c*) The number of chlamydospores always remained a few.	No fruiting occurred in any subculture.	The development of conidia in small numbers in these sub-cultures lends support to the persistence of the damage.

Changes in the second set of sub-cultures in tubes from the first set (*i.e.*, in the second vegetative generation)

(1) In the case of *Polystictus leoninus* in order to find out whether the damage in the first generation persisted and also to study the subsequent nature of growth, the last three subcultures of the first generation were again subcultured in malt-agar tubes on 1st December, 1936. These three subcultures constitute the second generation.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Smears from these tube subcultures showed a strong tendency to revert to the normal. In the first few smears damaged condition of hyphae was only slightly perceptible but this damage totally disappeared afterwards and complete reversion to the normal condition was found in this second generation. The damaged hyphae that were occasionally found formed nothing but a stage in the conversion of healthy living hyphae into dead double walled hairs as are found in old normal cultures. Hyphae were all with clamps and with mediate branches arising at right angles here and there, broad like the normal ones, with well-developed clamps. The broken up or vacuolated nature of the protoplasm totally disappeared. Out of the five sub-cultures from the main plate the last three were subcultured in wood block on thirteenth November, 1936. The woodblock cultures, therefore, represent the second generation. The wood	Fruits appeared in all these three sub-cultures after seven days which was also the fruiting period for the normal ones at the period. These fruits were quite normal with regular pores which showed in section basidia in close clusters bearing sterigmata and normal spores and were found intermixed with tramal hyphae bearing clamps but no spores (cystidia-like bodies.) Spore-fall was copious on agar plate whence they were transferred to malt-agar tubes and kept in incubator at 37°C owing to the then low temperature condition. The spores started germinating four days after they were transferred and regular fruits appeared after eight days (the normal period).	The woodblock cultures were made at a time when damage was fully persisting. in plates. But complete recovery to the normal condition was noted on the fourteenth January 1937 i. e.,forty-five days after wood was inoculated while fruiting occurred in all between 66-72 days after the inoculation i.e. between 21-27 days after recovery was noted, while the control culture on wood-block fruited between 14-36 days. The fruits formed were all very regular and all shed spores copiously in agar-plate on the next day after insertion. These spores germinated almost simultaneously and produced clamps after five days in agar plate. They were not transferred to malt agar tubes. The malt agar tube sub-cultures of the second generation were transferred to the third, fourth and fifth generations in tubes. In no case was any damage found to exist and fruits always occurred within the normal period. which was extended owing to the

In the vegetative stage.	In the reproductive stage.	Observations.
used was that of mango. Growth was very vigorous in wood and the very first smear examination on the fourteenth January, 1937 showed complete recovery of the vegetative hyphae to the normal state. (*b*) Conidia were very rarely met with. (*c*) Chlamydospores developed in small numbers in malt-agar tubes but they were not found in wood-block cultures.		low temperature conditions during the winter (10-12 days).

Changes in the second set of sub-cultures in tubes from the first set (*i.e.*, in the second vegetative generation)

(2) In the case of *Polyporus ostreiformis* the first three sub-cultures of the first generation were again subcultured in tubes and complete recovery of vegetative hyphae was noted in them.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Damaged nature of hyphae totally disappeared and smears showed normal hyphae with clamps and mediate branches. (*b*) Conidia were not found in any of them or in any subsequent generation. (*c*) Chlamydospores developed in good numbers.	No fruits appeared either in the second or third generation-subcultures as they were all in tubes (not fruiting in tubes then)	Damage was first noted in main plate on eleventh November,1936 and complete recovery was first noted in tubes of the second generation on twelfth December, 1936, the intervening period being 31 days. Subcultures were carried upto the third generation but no damage was found to exist. No transfers were made to wood-blocks.

Recovery from damage

In the case of *Polystictus leoninus* damage was first noted in the plate on 9th October, 1936, after the first exposure. It persisted throughout the first generation in plate subcultures and complete

recovery was noted in tube-subcultures of the second generation
on 9th December, 1936. The total period was therefore 60 days.
In wood-block cultures of the second generation the recovery period
calculated from the date of inoculation of the wood was 45 days.
The fruiting period of the main plate and of subcultures of
P. leoninus is summarised below :—

The main plate fruited in the course of 7 days. But no regular fruit-body or fruiting areas were formed.	Controls also fruited on the 7th day.
Sub-cultures Fruiting period	
No regular fruits formed in (1) and (2) (1) 1st generation fruited in 8—9 days.	Control fruited in 6-7 days
(2) 2nd. generation ,, ,, 7 days.	Control ,, on the 7th day.
Regular fruits formed in (3) (4) and (5) (3) 3rd. generation ,, ,, 10 days.	Control ,, also on the 10th day.
(4) 4th. generation ,, ,, 12 days.	Control ,, also on the 12th day.
(5) 5th. generation ,, ,, 10 days.	Control ,, also on the 10th day.

Recovery from damage in *Trametes cingulata* was noticed in the
first generation-subcultures and that in *Polyporus ostreiformis* in
the second generation.

Results (II) in the case of only two exposures of fifteen minutes each in a fortnight.

Damage in the main plate of *Polystictus leoninus*

(1) In the case of *Polystictus leoninus* two exposures were
altogether given, each of 15 minutes' duration. The first exposure
was given on 20th October, 1936, *i.e.*, 5 days after inoculation—
immediately preceding the usual fruiting date and the second exposure
on 5th November, 1936, *i.e.*, 15 days after the first exposure. The
irradiated plate together with a control was kept in diffuse light.
No heating was noted near the culture even after 15 minutes'
exposure. The vegetative hyphae were all healthy and there were no
basidia when exposure was first begun.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. After the first exposure dead empty or partially empty hyphae, some even with empty	Fruiting was retarded to a much greater extent than in the previous experiment. The first appearance of basidia was noted after	No tendency of the mycelial mat to go down to the bottom of the petridish was noted, neither was there any indication of

In the vegetative stage.	In the reproductive stage.	Observations.
clamps still attached, were found in large numbers and living hyphae altogether lost their healthy appearance and the protoplasm became broken up into chains of oidia showing a tendency towards haploidisation. During the next 15 days that intervened before the second 15 minutes' exposure, the vegetative growth was rather slow and the hyphae produced were both healthy or damaged in appearance. But the healthy hyphae, though filled up with protoplasm as those of the normal ones, were all much narrower than the normal ones and clamps much reduced in size. Hyphae without clamps which had the protoplasm disintegrated, were produced in large numbers but these were always exceeded by the numbers of hyphae with clamps which showed similar appearance. Many of these were found to be converted into dead, double-walled hairs. (*b*) Conidia were not found in the irradiated plate. (*c*) Chlamydospores appeared quicker (after 20 days) than in the case of daily 5 minutes exposure. The number of chlamydospores went on increasing from day to day till the second exposure after which a sharp decline in their number was noted. A large number of chlamydos-	15 days in smear only while the control fruited perfectly well after 8 days. Thenceforth, basidia could occasionally be found in smears, they never arranged in close series but always irregularly scattered and only rarely they were found with attached spores. Furthermore, the basidia were all much shrunken in appearance and much smaller in comparison with normal. No porous surface appeared and therefore, toothed areas were entirely absent. No outward indication of fruiting could be found from the a p p e a r a n c e o f culture as a whole except for the presence of one small area forming a yellow spot. This developed nearly after 25 days and did not develop any spores.	reversion to normal conditions in the main plate. The colour of the mycelial mat never changed from white to dull yellow or dirty yellow as was found in the previous experiment with the main plate.

In the vegetative stage.	In the reproductive stage.	Observations.
pores were killed by the second exposure, and some became empty, though living chlamydospores still persisted in good numbers. But these gradually diminished in number and almost vanished later.		

Damage in the main plate of *Trametes cingulata*

(2) In the case of *Trametes cingulata* the exposure was given on 10th November, 1936, and the second exposure on 26th November, 1936. Altogether two exposures were given, each of 15 minutes' duration.

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. The changes produced in the main plate were the same as in the previous experiment. The same nature of damaged hyphae was found. The second exposure only served to enhance the damage already present.	No fruit formed even in the control, as it was a non-fruiting strain.	Damage became evident after the first exposure on 10th November, 1936 and no sign of recovery was noted during the 15 days that elapsed before the second exposure.
(b) Conidia were enormous in the beginning, but they slightly diminished till the second 15 minutes' exposure. The conidia disappeared altogether after the second exposure of 30 minutes.		
(c) Chlamydospores were present in small numbers throughout but they too disappeared altogether after the second exposure.		

(3) Damage in the main plate of *Polyporus ostreiformis*

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In the case of *Polyporus ostreiformis* damage was evident after the very first exposure. A good number of hyphae were killed and emptied out and many of the living hyphae showed protoplasm broken up into disconnected chains. During the fifteen days that followed before the second fifteen minutes, exposure a slight tendency to revert to the normal state was noted in the irradiated plate, as the number of healthy clamped hyphae slightly increased from day to day. The second exposure caused considerable damage to the vegetative hyphae as healthy hyphae almost disappeared from the culture. Many more hyphae were emptied out and lost their clamps altogether; out of the living ones a good number was found to undergo disintegration. After the second exposure a good number of long, uniformly narrow and filled-up hyphae without clamps developed mixed with damaged clamped hyphae. Clamped hyphae that remained were all damaged in appearance and were either extremely narrow or some as broad as normal. The number of mediate branches	Fruit appeared in the irradiated plate on 25th. November, 1936, i. e. 20 days after the inoculation and immediately preceding the second exposure on 26th. November 1936, on the wall of the plate. The control plate fruited one day earlier on 24th. November 1936. The fruit in the irradiated plate showed regular pores and a section showed pure basidia in dense layers in each poretube, bearing sterigmata and spores exactly similar to the normal spores in length and breadth. The fruit was inserted in agar plate on 12th December, 1936. Sporefall was copious and continued for three days. The agar plate was kept inside the incubator. Germination was quick and in normal manner and a smear-examination after five days showed normal hyphae of *Polyp. ostreiformis* with clamps, mediate branches and chlamydospores.	As the fruit was formed after the first exposure it is evident, therefore, that continuous exposure for 15 minutes caused damage only to the vegetative phase and did not at all affect the reproductive phase in *Polyp. ostreiformis*.

In the vegetative stage.	In the reproductive stage.	Observations.
neither increased nor decreased.		
(*b*) Conidia, a few in number were developed on 2nd December, 1936 after the second exposure (27 days after inoculation) but they never increased.		
(*c*) Chlamydospores were in good numbers in the beginning but they diminished in number after the second exposure and never vanished altogether.		

Changes in subcultures from the main plate (*i. e.* in the first vegetative generation)

(1) In the case of *Polystictus leoninus* altogether three sub-cultures were carried out from the main plate. The first subculture was after the first 15 minutes' exposure, the second after the second 15 minutes' exposure, and the third 20 days after the second exposure. Of these three subcultures, the first and the second were in plate while the third was in tube.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The characters of the first two subcultures were almost identical, but the third subculture differed in many respects. In the first two subcultures the damaged conditions of the vegetative hyphae fully persisted. They were all much narrower than the normal ones with clamps extremely small and reduced in size. Protoplasmic discontinuity of the hyphae with a strong tendency to liberate oidia was evident.	Fruits appeared in all after 9 days, while the fruiting period in the control (at that time) was 6-7 days, so that fruiting was delayed by 2-3 days. The fruits on sectioning showed regular pore-tubes within which were found a large number of basidia in series with attached basidiospores, probasidia and tramal hyphae bearing clamps only (cystidia-like). The fruits of the third subculture in sections showed regular pores containing basidia,	It is thus seen that though considerable damage persisted in two plate sub-cultures of the first generation a small amount of reversion to normal state was also noticeable in the first generation since (a) the condition of the vegetative hyphae in the tube-sub-culture was almost like that of the normal and the damage was only slight; and (b) the fruits of the first generation (both in plate and tube) developed regular pore-

In the vegetative stage.	In the reproductive stage.	Observations.
Clamped and non-clamped hyphae were both present; some-times non-clamped ones were in excess of the clamped ones while at other times the clamped ones were greater in number. Mediate branches were always present and conversion of living hyphae into hairs was noticeable to the same extent as in the main plate. Another feature was that the damaged condition of hyphae was always more pronounced in the second subculture (made after the second exposure) than in the first one. The third subculture in tubes twenty days after the second 15 minutes exposure was quite unlike the other two in that the damaged condition of the vegetative hyphae was only slightly perceptible. Damaged hyphae were some-times found but the majority of the hyphae were much healthier though still much narrower than the normal ones. Majority were filled up and mostly arranged in parallel rows in smears.	basidiospores and tramal hyphae elongat-ions. Two fruits from the second subculture and another from the third subculture were inserted in agar plate on 12th December, 1936. Spore-fall was scanty on the next day from both but spores did not germi-nate even after 7 days. As it was thought that germination was checked owing to crowding of spores in a limited space, spores were transferred from both to malt agar tubes and kept inside incuba-tor (37°C) owing to low temperature conditions at that time together with the agar-plate containing spores. But none of the spores germinated. The spores were not, therefore, viable.	tubes, containing basi-dia and basidiospores though the latter were non-viable.
(*b*) Conidia in the first two subcultures were almost none, one or two being very rarely found, while in the third subculture conidia were in fairly good number.		
(*c*) Chlamydospores in the first two subcul-		

In the vegetative stage.	In the reproductive stage.	Observations.
tures never developed in large numbers and their number remained a few throughout; a peculiar feature was the formation by s o m e cystidia-like chlamy-dospores. But in the third s u b - c u l t u r e c h l a m y d o s p o r e s developed enormously.		

(2) In the case of *Trametes cingulata* three subcultures from the main plate were carried out the first after the first 15 minutes' exposure, the second after the second 15 minutes' exposure and the last on the twelfth day after the second exposure. Complete recovery to the normal state was noted in all of them. Subcultures were carried up to the third generation and in no case damage was found to persist. Damage was noted first on 10th November, 1936, and recovery on 23rd November, 1936, thus the intervening period was 13 days. Exposures given daily for 5 minutes are, therefore, more injurious than a single long exposure twice in a fortnight. No transfers were made to wood-block and fruiting occurred anywhere even in the control.

(3) In the case of *Polyporus ostreiformis* three subcultures were carried out from the irradiated plate, the first after the first 15 minutes' exposure, the second after the second 15 minutes' exposure and the third and the last nine days after the second exposure. The first two subcultures were in plates but the last one was in tube and hence no question of fruiting can arise in the case of the last subculture. But while the last subculture showed *complete* reversion to the normal state, the first and the second subcultures showed some differences.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In the first subculture the hyphae were slightly damaged and majority were healthy, while in the second ones majority of the hyphae were damaged and only a few were healthy.	No fruiting occurred in the second sub-culture (i.e., after 30 minutes's exposure). But fruiting occurred in the first subculture (i.e., after 15 minutes's exposure) within the normal period—nineteen days after inoculation. The fruit was	Only the last sub-culture showed *complete* reversion to the normal state.

In the vegetative stage.	In the reproductive stage.	Observations.
(*b*) No conidia were present in the first sub-culture, but a few conidia could be found in the second. (*c*) Chlamydospores were a few in the first sub-culture but always greater in number in the second.	well-formed showing in cross-section pure basidia bearing sterigmata and spores fully agreeing with the normal ones in measurement. The fruit was inserted in plate on 23rd December, 1936, and spore-fall occurred within four hours and continued for five days. The spores germinated quickly inside the incubator at 37° C. in normal manner and clamps developed after five days in agar plate.	

Changes in the second set of sub-cultures in tubes from the first set (*i.e.*, in the second vegetative generation)

(1) In the case of *Polystictus lconinus* as growth became checked in both plate and tube-subcultures they were transferred on 2nd December, 1936, to fresh malt-agar tubes. They formed the second generation. The damage totally disappeared and complete reversion to the normal state took place in the second generation.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The hyphae were all like the normal ones and were filled up with well-developed clamps and mediate branches. The three subcultures from the first generation (in plate) were transferred to wood-blocks (mango) on 11th November, 1936. Growth was very vigorous and smear examination on 2nd January, 1937 showed complete recovery in wood-blocks too. Recovery, therefore, took place after thirty-three days in all.	In malt-agar tubes fruits appeared on 9th December 1936 within the normal period (7 days). The fruits on sectioning showed basidia with attached spores mixed with tramal hyphae bearing clamps and some even bearing terminal mature spores. Three fruit each from one sub-culture were inserted in agar plate on 18th December, 1936. Spore-fall occurred on the next day from all but their number was scanty in comparison with the large number of spores discharged	Sub-cultures were carried upto the fifth generation. Damaged condition was never found and fruiting always occurred within the normal period which ranged from 10 to 11 days.

In the vegetative stage.	In the reproductive stage.	Observations.
(b) In malt-agar-tubes no *conidia* were produced as in the case of normal growth, but in wood block cultures a small number of conidia was found. (c) In both cases (malt-agar tubes and wood-block culture) a few *chlamydospores* were found.	from a normal fruit. However, the spores all germinated inside the incubator after four days whence they were transferred to malt-agar tubes. Among the wood-block cultures fruit formation occurred in the first subculture on 10th April, 1937, after 120 days; in the second subculture on 21st April, 1937, after 131 days; and in the last subculture on 4th February 1937 after 55 days. The fruiting period, when calculated from the date of recovery, was as follows :— First subculture 　　　　87 days Second subculture 　　　98 days Third subculture 　　　22 days Control 14-36 days The fruits were all well-formed with regular pores and were inserted in agar plate. Spore-fall was obtained by the next day upon agar plate, spores germinated in normal manner and clamps developed after five days in agar plate. They were not transferred to malt-agar tubes.	.

(2) In the case of *Polyporus ostreiformis* though complete recovery was noted in the last subculture (in tube) of the first generation, recovery was partial in the first two subcultures. They were, therefore, again subcultured in tubes on 8th December, 1936, and complete recovery was noted. But as they were in tubes and not in plates, no fruit appeared. Subcultures were carried upto the third generation in tubes and no damage was noticed in any case. No transfers were made to wood-block.

Recovery from damage

In the case of *Polystictus leoninus* damage was first noted in the main plate after the first exposure on 21st October, 1936. The damage persisted in plate subcultures of the first generation. Complete recovery was noted in tube subcultures of the second generation on 9th December, 1936. The total period was 44 days. The recovery-period in wood-blocks (which represent cultures of the second generation) calculated from the date of inoculation of the wood was 33 days. Daily five minutes' exposure is, thus, more injurious than one long exposure given twice. Ramsey and Bailey (13) also hold that "increasing the number of exposures is more effective than increasing the length of exposure". The fruiting period of the main plate and of the subculture of *P. leoninus* is summarised below :—

	The main plate fruited in the course of 15 days. But no regular fruit-body or any fruiting areas nor any spores were formed, only Lasidia had appeared.	Control fruited in 8 days
	Subcultures Fruiting period	
No Regular fruits formed in (1) & (2)	(1) 1st. generation 9 days	Control fruited in 6 - 7 days
	(2) 2nd. generation 7 ,,	Control fruited in 7 days
Regular fruits formed in (3) (4) & (5)	(3) 3rd. generation 10 ,,	Control ,, 10 days
	(4) 4th. generation 11 ,,	Control ,, 11 day
	(5) 5th. generation 10 ,,	Control ,, 10 days

Recovery from damage in both *Trametes cingulata* and *Polyporus ostreiformis* was noted in the first generation subcultures. In *Polyporus ostreiformis* damage was first noticed in the main plate after the first exposure on 11th November, 1936. Complete recovery was noted in the third tube-subculture of the first generation on 6th December, 1936. The interval, therefore, was 25 days, which is quicker than in the previous case.

Leaving aside fruit-formation in the main plate which occurred after the first exposure of 15 minutes at a time when there was no great damage to the hyphae, fruit-formation in the first subculture from the main plate deserves notice. This subculture was carried out when the main plate had received continuous exposure for 15 minutes and the fruit formed was normal in every respect. But the corresponding subculture in the previous experiment after three consecutive exposures of 5 minutes each, showed no fruiting. It is, therefore,

evident that even as far as fruit-formation is concerned (*i.e.,* in the reproductive phase), exposures of 5 minutes' duration given on three successive days are more injurious than a single long exposure of equal duration. This is further supported by the fact that in every case studied recovery in the vegetative phase was quicker in the case of twice 15 minutes' exposure in a fortnight than that of daily 5 minutes' exposure for 15 days. This statement applies equally well to *P. leoninus, T. cingulata* or *P. ostreiformis.*

Irradiation of fresh spores of *Polystictus leoninus* by ultra-violet rays (a) for thirty minutes (b) ten minutes and (c) five minutes.

(*a*) A sporophore of *Polystictus leoninus* from a tube culture was inserted on agar plate on 27th January, 1937. Spore-fall occurred within four hours of insertion and was collected in two plates. One plate containing fresh ungerminated spores was irradiated by ultra-violet rays through cellophane paper (·025 mm. thick) on the same day for a period of *thirty minutes* while the other plate was kept as a control. The type of lamp (Hanovia Alpine Sun), the distance from the arc etc. were the same as in the previous experiments. Both the irradiated and non-irradiated plates were kept inside the incubator at 37° C. The control spores germinated within three hours while the irradiated spores did not germinate even after eight days.

(*b*) Another agar plate containing fresh spores of *Polys. leoninus* dropped from an artificial fruit on 3rd February, 1937, was similarly irradiated for *ten minutes* keeping a control plate. Both the plates were kept inside an incubator at 37° C. The spores in the control-plate all germinated by the next day while the irradiated spores did not germinate even after eight days. Some of the irradiated spores were transferred to malt-agar plate, but here they also did not germinate.

(*c*) On 23rd February, 1937, fresh spores of *P. leoninus* in agar plate were similarly irradiated for five minutes only, keeping a control plate. Both plates were kept as before inside the incubator at 37° C. The spores in the control-plate all germinated by the next day while a good number of irradiated spores germinated only after two days, *i.e.,* on the 25th February; they were both subcultured to malt-agar tubes and were kept in the culture room. Growth was vigorous in the tubes containing normal (non-irradiated) spores and perfect fruits appeared in the course of seven days while the tubes containing irradiated spores showed no sign of progress at all even after sixteen days.

On the same day fresh spores in an agar-plate from an artificial fruit of *P. leoninus* in a tube culture after complete recovery from the effects of radium (fruit of the third subculture in the fourth generation from the main plate of *P. leoninus* exposed to 120 mg. of radium for six days) were irradiated for five minutes only,

keeping a control-plate. The spores in the control-plate all germinated by the next day while the irradiated spores germinated two days later. Both were transferred to malt-agar tubes and were kept in the diffused light of the culture-room. As in the preceding case growth was vigorous in the tube containing non-irradiated spores, leading to the formation of perfect fruit in the course of seven days, but the tube containing irradiated spores did not show any trace of growth even after sixteen days. Thus, it is seen that spores dropped from a sporophore of *P. leoninus* in malt-agar tube after complete recovery from treatment with heavy doses of radium reacted to ultra-violet rays in the same way as spores from a normal fruit-body in culture.

Side by side, the influence of sunlight, incandescent light (150 C. P. bulb) and of heat upon cultures of these Polypores was studied.

Influence of sunlight exposure for ten days on culture of (a) Polystictus leoninus.

A plate culture of *P. leoninus* inoculated on 25th November, 1936, was exposed to solar radiation. The petri-dish containing the culture was placed on a thick glass plate over which was placed a belljar the rim of which was sealed to the glass surface with vaseline. It was exposed to the direct rays of the sun over a raised horizontal wooden platform. Exposure was first given on 28th November, 1936, *i.e.*, three days after the inoculation and ended on 9th December, 1936. During this period exposures were given almost daily for 10 days. The period of exposure was four hours from 12—4 P.M. The temperature of the surrounding air varied from 30°–33° C. Two subcultures were carried out, the first after 3 days' exposure and the second after 6 days' exposure.

Results in the main plate

The culture media contracted from the edges of the plate and gradually came to occupy the centre with increasing evaporation of moisture, it became reduced to a thin sheet and presented a dried and hardened appearance. The result produced by solar radiation was much more significant than that produced by ultra-violet, X-ray or radium exposures. The very first exposure on 28th November, 1936, was sufficient to check the growth of the colony. No further growth occurred during the period before the second exposure and growth became permanently checked. The fluffy nature of the hyphae became entirely lost and the whole hyphal mat presented an adpressed appearance but did not tend to sink down to the bottom.

With increasing exposures the hyphae became extremely narrow with clamps extremely reduced in size. The protoplasm became broken up into disconnected masses with a tendency to break up into oidia. A few conidia developed after the third exposure on 2nd December, 1936 *i.e.*, 4 days after inoculation but they never increased. Furthermore, the number of mediate branches became

very much reduced. A large number of hyphae became totally clampless and such non-clamped hyphae came into preponderance. Hyphae were also found to disintegrate in large numbers. No chlamydospores were produced. There were no basidia when the culture was first exposed, and no basidia ever developed in the main plate, so that fruiting was entirely suppressed. In other words, the culture ultimately became dead. Hot-agar was poured over the culture on 15th December, 1936 but there was no revival.

Results in the sub-cultures (*i.e.*, in the first vegetative generation)

Two subcultures were carried out in malt-agar tubes after the third and sixth exposures, but no growth was noticed at all either at room temperature or in incubator at 37° C.

Influence of sunlight-exposures for fifteen days on culture of (b) *Trametes cingulata* :—

A petri-dish culture of *T. cingulata* inoculated on 4th May, 1937, was exposed after six days to direct solar radiation. The plate was almost full when the culture was first exposed. Exposures were given for three hours daily for 15 days, the first exposure being given on 10th May, 1937, and the last on 26th May, 1937.

The time of exposure was from 9 A.M.–12 P.M. The temperature of the surrounding atmosphere varied during this period from 35°–40° C. The method of exposure was the same as in the previous experiment, excepting that the sides of the petri-dish were covered by a strip of white paper, and an Erlenmeyer flask containing 4% alum solution was placed over the petri-dish inside the belljar. This devise eliminated a certain amount of heat and the temperature taken by actually inserting a thermometer inside the petri-dish during exposure was found to be 30°–35° C. which was less by 4 to 5 degrees than that of the surrounding atmosphere and higher by 1 or 2 degrees than the room temperature.

Results in the main plate

(1) The first effect was a change in the external appearance of the culture. The culture media together with the hyphal culture contracted gradually from the circumference of the petri-dish and ultimately came to occupy about three-fourths of the plate owing to evaporation of moisture from the medium. The thickness of the medium was also very much reduced. The plate showed distinct zonation before exposure—the effect of alternation of light and darkness—but the difference between zoned and non-zoned areas ultimately disappeared and the culture presented an adpressed appearance.

(2) Damage to vegetative hyphae was rather insignificant in comparison with *P. leoninus.* A good number of dead and empty

hyphae were produced. The protoplasm of vegetative hyphae became broken up into disconnected chains and a strong tendency to break up into oidia was noticed at places only. But the majority of the hyphae were healthy though a little narrower than the normal ones.

(3) The number of conidia slightly increased at first but after the third exposure, they *rapidly diminished* in number as the majority of them became converted into *thick-walled chlamydospores.* Chlamydospores ultimately preponderated over conidia.

(4) Mediate branches, in addition to those already present, were found to increase. They were not produced from the main hyphae, but were developed as direct prolongations of the clamps. They were short in length, without any clamps, and did not present a damaged appearance. This is usually regarded as reduction to the monocaryon stage, as pointed out by Brodie (3). No fruit appeared as it was a non-fruiting strain.

Results in the sub-cultures (*i.e.,* in the first vegetative generation)

Three subcultures were taken from the main plate, the first after the second exposure on 12th May, 1937, and the second after the seventh exposure on 18th May, 1937, and the third after the fifteenth exposure on 27th May, 1937, in malt-agar tubes. Complete recovery was noted in all these subcultures in the course of four days, conidia developed in large number and nowhere damage was found to persist. Wood-block *(Acacia)* subcultures were taken from the main plate and from the second subculture of the first generation on 28th May, 1937. The wood-block culture from the main plate represents the first generation while that from the second subculture represents the second generation. The first smear taken from both the wood-blocks on 4th June, 1937, *i.e.,* seven days after inoculation, showed complete recovery to the normal state. Growth was very vigorous in both. No fruiting appeared anywhere as it was a non-fruiting strain.

Influence of incandescent light (150 c.p. bulb)

(A) On culture of *Polystictus leoninus.*

A petri-dish culture of *P. leoninus* inoculated on 10th August, 1936, was exposed to incandescent light from 15th August (*i.e.,* five days after inoculation) to 5th September, 1936. During this period altogether sixteen exposures were given, each exposure being of 6 to 7 hours duration. The culture was placed at a distance of 50 cm. directly below the source of illumination. The temperature recorded near the culture varied from 30° to 32° C. and this was always found to be higher by only one degree than the room temperature. The petri-dish lid was never replaced by cellophane so that a portion of the light was cut off by the glass. Exposures were always given in a dark room thus excluding all chances of outside illumination.

Results in the main plate

The hyphae were all binucleated with, as usual, a good number of mediate branches and a few chlamydospores before exposure was begun. There were no conidia and no trace of basidia.

(1) *Damage to the vegetative hyphae* by such long exposures to strong illumination was not much. The protoplasm of the vegetative hyphae gradually fragmented. A large number of hyphae were devoid of cell contents, lost their clamps and became converted into hairs. But this is quite a normal phenomenon and takes place in normal culture as it becomes old. No tendency to break up into oidia was noted. No conidia developed and the number of chlamydospores was never found to increase.

(2) *Damage to the reproductive stage.*

The first appearance of basidia was noted in smear only on 18th August, 1936, *i.e.,* 8 days after inoculation while the control fruited perfectly well after 6 days. The basidia were all immature, though present in good number. They were never found to be arranged in close cluster, but were always irregularly scattered. They never came to maturity and were never found to bear sterigmata or spores. The basidia, however, became much affected by radiation. They gradually became much shrunken in appearance, decreased in number and totally disappeared from the culture after the tenth exposure on 28th August, 1936. No fruiting area was formed upon the culture and no porous surface was formed, so that fruiting could not be detected from the appearance of the culture as a whole.

No further subculture or transfers to wood-blocks were carried out.

(B) On culture of *Polyporus ostreiformis.*

A petri-dish culture of *P. ostreiformis* inoculated on 10th August, 1936, was exposed in the same manner to incandescent light from 15th August, 1936, to 5th September, 1936. The details of the method were the same as in the previous experiment. Altogether sixteen exposures were given, each exposure lasting for 6 to 7 hours.

Results in the main plate

(1) *Damage to the vegetative hyphae* was slight. The hyphae were all binucleated, broad and narrow, with a good number of mediate branches when exposure was first begun. A few chlamydospores were present. This condition continued right upto the end but as the culture became old, the number of chlamydospores was found to increase and a good number of vegetative hyphae had protoplasm fragmented at places only. Some empty hyphae were also produced. But these are normal phenomena and cannot be attributed to the effect of strong illumination.

(2) *Damage to the reproductive phase* was very great. No fruiting ever occurred in the main plate while the control plate fruited perfectly well after 18 days. No moist spot developed in the main plate anywhere and the presence of basidia could not be detected even in smears.

No further subcultures or transfers to wood-blocks were carried out.

Influence of heat upon culture of *Trametes cingulata*

A full-grown plate of *T. cingulata* was exposed to heat. Half of the plate was placed over paraffin bath at 55° C. while the other half was projecting out and was held in position by clamp. The plate was heated for 5, 10, 20 and 30 minutes, but no damage was noticed either to the hyphae or to the conidia. Heating was then continued for 40 minutes. It was observed that some clamps had fallen out and that empty hyphae were found in fairly large number and conidia were killed in number. The control half was normal. The heating effect slowly passed away and the heated half gradually reverted to the normal state within two to three days, as revealed on smear-examination.

Another full-grown plate of *T. cingulata* was treated on the same day with X-rays at 150 K V., 3 m. a. at 30 cm. from the target for fifteen minutes. Half of the plate was exposed to X-rays while the other half which served as control was screened with lead rubber. On comparison damage was found to be greater with X-rays than with heat. Barnes (1) also held that X-rays alone were much more effective than increases of temperature.

Ultra-violet and Solar Radiations
Discussion and conclusion

Though in all three cases (*Polys. leoninus, T. cingulata* and *Polyp. ostreiformis*) there was finally reversion to the normal state within a varying period, it was clear from the foregoing results that increasing the number of exposures was more effective in causing damage both to the vegetative and reproductive phases than increasing the length of exposure, as held by Ramsey and Bailey (13). In the case of *Polyporus ostreiformis* the greatest damage to the reproductive phase (fruit-formation) was caused by ultra-violet rays of daily five minutes' exposures for fifteen days with the first vegetative generation subcultures, where no fruiting areas or pores or any basidia were found; whereas in the main plate irregular fruiting areas without pores and rudimentary basidia without spores were found. This might probably be due to the "latent period" or to the delay in the appearance of the effects of radiation in living organisms. In the case of *Polystictus leoninus*, however, the greatest amount of damage to the reproductive phase was caused in the main plate, where no porous surface and no erect fruit-body were formed though a few shrunken basidia were irregularly scattered without attached spores. In the first vegetative generation subcultures from the main plate exposed to daily radiation of five minutes for

fifteen days a porous surface was formed with basidia arranged in series. These basidia were without attached spores and in the final erect fruits the pore-tubes were empty. But in the first vegetative generation subcultures from the main plate, exposed to only twice fifteen minutes' exposures in a fortnight, erect fruits were formed with regular pores and basidia arranged in series with attached spores, but the sporefall in moist agar plate was scanty and spores were not viable. Normal viable spores from normal erect fruit-bodies were found only in the second generation subcultures where complete recovery was noticed.

The effect of exposure to direct sunlight minus ultra-violet rays but combined with higher temperature was much more pronounced than that of ultra-violet, X-rays or radium in both the vegetative and reproductive phases. The killing effect was evident after the second exposure and the cultures ultimately died, they could not be revived in subcultures ; thus, the fruit formation was permanently checked. In the case of incandescent light (150 c.p. bulb) however, the damage to the vegetative hyphae was slight though in the reproductive phase the fruit-formation was ultimately suppressed. These Polypores are white without any pigment. Burkholder (4) has remarked that "where light exerts an action upon growth, it is probably brought about by absorbing substances (pigments) present in plants." Exposing culture of *Trametes cingulata* to heat (55° C.) for forty minutes some damage was produced, which however, passed off in the course of two to three days. Dickson (7) could get no saltation by application of heat. Comparison with X-rays shows that X-rays produce greater damage than heat alone. Ultra-violet rays had great killing effect on freshly shed spores on moist agar surface, with decrease of exposure to only five minutes some of the spores could germinate but they could not progress further. Stevens (17) also found that an exposure of ten minutes to ultra-violet rays killed the spores. My experimental results confirm the observations of Smith (16) that " the changes in fungi which have been described as due to the influence of visible and ultra-violet light have not been heritable. They have been in no sense mutations."

One common feature of these different modes of treatment was almost universal reduction in fertility, sometimes the action of the external agent was so violent as to cause the death of the organism or of the spore, that is, in such cases the damage has been so severe that it cannot be repaired. In other cases the damage has been of a temporary nature so that there is ultimate reversion to the normal form after a period of growth. Barnes (1) is probably right here in holding that treatment with violent external agents may well hasten the slow normal changes of a degenerative character, bringing about a general derangement of physiological balance of the cell. Karl Sax (14) holds that "heat and age seem to induce the same effects as X-ray treatment."

Influence of X-rays on artificial cultures of three Polypores

Two kinds of X-rays were employed—(1) hard rays from a Coolidge tube of 150 K V. potential with tube-current of 3 milli amperes, the distance from the target being 30 cm. and (2) soft rays of 50 K. V. and 30 K V. potential with tube-current of 2 milli amperes and 3–5 m. a., the distance from the target being 18 and 10 cm. respectively.

Daily one hour (hard) X-ray exposure for 14 days at 150 K V., 3 m. a. on cultures of *Polystictus leoninus* and *Polyporus ostreiformis.*

Damage in the main plate

(1) A malt-agar plate of *Polys. leoninus* inoculated on 25th June, 1936, was taken. The culture received 14 exposures in all on almost consecutive days. With the exception of the first four exposures which were given for 30 minutes each, the remaining ten exposures were each of one hour's duration. Exposure was first begun on 1st July, 1936, *i.e.,* 5 days after inoculation and ended on 28th July, 1936. The petri-dish lid was replaced by cellophane (sterilised with alcohol) during exposure. The hyphae were with clamps and some mediate branches, they were non-conidial without any trace of basidia. A plate culture was kept as a control.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae The first exposure to x-rays produced very appreciable damage to the protoplasm and heavy damage was noticed after the fifth e x p o s u r e. The changes produced in the vegetative hyphae were death and destruction. With increasing doses of radiation the protoplasm of living hyphae became more and more damaged and showed a very much vacuolated appearance or were in disconnected chains with a strong tendency to break up into oidia (i.e. conversion to the monocaryon stage). Clamps were destroyed in good many hyphae	Fruiting was greatly affected in the main plate. The first appearance of basidia was noted on 5th July (1936), i.e. 10 days after inoculation while the control plate produced perfect fruit on the 7th day after inoculation, so that fruit-formation was delayed by 3 days in the irradiated plate. The basidia at their first appearance were all in dense cluster but they did not bear sterigmata or spores, tramal hyphae bearing clamps but with no terminal spores were sometimes found amongst the basidia. Some abortive fruit-bodies appeared in the main plate after 15 days and these on sec-	No saltant hyphae were produced. Not only fruit-formation was delayed by 3 days, but the development of reproductive bodies was almost entirely suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
and numerous extremely narrow non-clamped hyphae in addition to mediate branches were produced. But the number of clamped hyphae (dicaryon ones) preponderated. Empty hyphae and hairs were produced in large number. (*b*) A small number of conidia appeared at the end on 28th July 1936, i.e. 34 days after inoculation. (*c*) Chlamydospores which at first were few, were found to increase in large numbers later on.	tioning several days after they had been formed, were found to consist only of hairs, not even any pores were formed. Basidia were scarcely visible on smear examination, they were few and irregularly scattered and very much shrunken.	

(2) A malt-agar plate was inoculated with *Polyporus ostreiformis* on 25th June, 1936, and was exposed to X-rays. The lid of the petri-dish was replaced by cellophane during exposure. Exposure was first given on 30th June, 1936, *i.e.,* 5 days after inoculation and ended on 27th July, 1936. During this period altogether 14 exposures were given. With the exception of the first four exposures which were given for 30 minutes each, all other exposures were of one hour's duration. The hyphae were all broad with clamps, measuring $4-6\mu$ in breadth, with a good number of mediate branches and chlamydospores, they were non-conidial when the exposure was begun. A plate-culture was kept as a control.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae Damage to the vegetative hyphae was first noticed after the third exposure on 3-7-36, the first and the second exposures having no effect. The damage was very slight, the	No fruiting ever occurred in the irradiated plate, though the control plate fruited perfectly well on 14-7-36 (after 19 days). A moist spot developed on the wall of the irradiated plate on 18-7-36 (23 days) but this did	Fruit-formation was entirely suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
protoplasm of living hyphae having become broken up at places only. With increasing exposures, the damage became more and more pronounced. Vacuolation went on increasing resulting in the breaking up of the continuous mass of protoplasm into disconnected masses. In many hyphae, the protoplasm contracted from the cell-wall and presented a streaked appearance, showing a tendency to breaking up into oidia. A large number of hyphae became dead and empty and many such empty hyphae with clamps partially or totally empty but still attached, could be found. Narrowing of hyphae was another feature which became evident after increasing exposures and broad healthy hyphae totally disappeared. (*b*) A few conidia developed after the 13th exposure, i.e. 28 days after inoculation. But their number never increased. (*c*) The number of chlamydospores became more and more reduced till they disappeared entirely at the end.	not develop any further and did not show any differentiation into porous area. A smear examination from this moist spot showed a good number of hyphae with swollen club-shaped ends suggestive of rudimentary basidia.	

Changes in sub-cultures from the main plate (*i.e.*, in the first vegetative generation)

(1) In the case of *Polystictus leoninus* altogether six subcultures were carried out from the main plate; the first after the first X-ray exposure on 1st July, 1936, and the second, after the third exposure

on 3rd July, 1936. The rest became contaminated and were, there-
fore, rejected. The smear-examination of these two subcultures
gave almost identical results.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae The protoplasm of vegetative hyphae presented a much damaged outlook and was much vacuolated or in disconnected chains. The hyphae became all narrower than the normal, and extremely narrow hyphae could be found. Empty hyphae without clamps and living hyphae with totally or partially empty clamps were found in large number and all of them became ultimately converted into dead double-walled hairs. But always clamped hyphae preponderated over the non-clamped ones. (b) Conidia in non-clamped hyphae appeared in the first sub-culture after 27 days and in the second sub-culture after 25 days in large numbers. (c) Chlamydospores appeared in large numbers in both the first and second subcultures.	Irregular fruit-bodies appeared in both the subcultures after ten days as could be seen from the appearance of toothed areas round the peripheral regions of the cultures, the control fruited on the eighth day. Basidia developed in large number at first but they were always irregularly scattered among masses of dead double walled hairs with neither sterigmata nor spores; a few tramal hyphae with only clamps were intermixed with them. The basidia were of irregular shape and appeared very much shrunken even at their first appearance. As the cultures grew older, the basidia disintegrated and disappeared.	As in the main plate not only fruiting was delayed but the formation of regular perfect sporophores was altogether suppressed.

(2) In the case of *Polyporus ostreiformis* altogether five subcultures were carried out from the main plate; the first after the first exposure, the second after the third exposure, the third after the sixth exposure, the fourth after the eighth exposure, and the fifth after the fourteenth or the last exposure. All these subcultures were in malt-agar plates.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. All s u b c u l t u r e s showed the damaged character of the vegetative hyphae, but the amount of damage was far greater in the last two subcultures than in others. (*b*) No conidia or any dead hyphae were found. (*c*) Chlamydospores were present in all in fairly good number.	No fruiting occurred in any plate but hyphae with swollen ends suggestive of rudimentary basidia could be found intermixed with damaged hyphae in all plate-cultures.	As in the main plate the reproductive phase was totally checked. No moist spot ever developed on the wall of the plates.

Changes in the second set of sub-cultures from the first set (*i.e.*, in the second vegetative generation)

(1) In the case of *Polystictus leoninus* two subcultures from the main plate were again subcultured in plates on 5th August, 1936.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Very few non-clamped hyphae were present and the condition of the protoplasm resembled normal. (*b*) Conidia were produced in moderately good number. (*c*) Chlamydospores were also found in good number.	Fruit-body appeared in both these sub-cultures after eight days (*i.e.*, 13th August, 1936) and this was the fruiting period for the normal at that time. Toothed areas showing regular pores developed and a section showed a few basidia intermixed with a few tramal hyphae bearing clamps.	It is thus found that with the exception of suppression of spore-formation there was definite reversion of the vegetative hyphae to the normal condition, and possibly complete reversion would have occurred if the plates were again subcultured as soon as they were filled up.

(2) In the case of *Polyporus ostreiformis*, the last two subcultures showed a greater amount of damage than others. They were again sub-cultured in malt-agar tubes on 1st August, 1936. A smear-examination on 4th August, 1936, showed complete recovery of damaged hyphae to the normal state. But no fruit-body appeared in them as they were in tubes and not in plates, no fruit-body having occurred at that time in tubes even in controls.

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Recovery from damage

(1) In the case of *Polystictus leoninus* damage appeared in the main plate after the first exposure on 1st July, 1936. Recovery of the vegetative hyphae was noted in both the subcultures of the second vegetative generation on 10th August, 1936, *i.e.,* after 39 days.

Fruit-body appeared		Control
In Main plate	in 10 days	Fruited in 7 days
In Subcultures :		
(*a*) 1st. generation	,, 10 days	,, ,,
(*b*) 2nd. generation	,, 8 days	,, 8 days

(2) In the case of *Polyporus ostreiformis,* damage to the vegetative hyphae occurred first after the third exposure on 3rd July, 1936. Recovery of the vegetative hyphae was noticed first in tube subcultures of the second vegetative generation on 4th August, 1936, *i.e.,* after 32 days.

Daily 15 minutes (hard) X-ray exposure for seven days at 150 K V. with three milli amperes on cultures of *Polystictus leoninus* and *Trametes cingulata*

(1) A malt-agar plate was inoculated with *Polystictus leoninus* on 9th June, 1936. The culture was exposed to X-rays from a Coolidge tube at 150 K V. 3 m. a. for seven days for fifteen minutes on each day. The first exposure was given on 13th June, *i.e.,* 4 days after inoculation, and the last, on 22nd June, 1936. The petri-dish lid was replaced as before by cellophane sterilised with alcohol. There were no basidia when exposure was begun, the strain showed hyphae with clamps and with a number of mediate branches and there were no conidia or chlamydospores or dead double-walled hairs. A separate malt-agar plate-culture was kept as a control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The changes in the vegetative hyphæ were that they became more and more vacuolated, and with increasing	The first appearance of basidia was noted on the eighth day after inoculation (i.e. on 16th June) which falls within the normal p e r i o d. Regularly	The chief peculiarity of the damage was that mature spores were never found on the sterigmata and t h e basidia were of an irregular type like an

In the vegetative stage.	In the reproductive stage.	Observations.
exposures they lost their clamps and protoplasmic contents and were ultimately converted into d e a d double-walled hairs. Non-clamped hyphae w i t h protoplasm broken up into pieces were produced in good numbers but they were only stages in the formation of hairs. No narrow hyphae were produced. It was found on the whole that clamped hyphae preponderated over the non-clamped o n e s . This type of changes was also noticed when a normal culture becomes old, thus the damage produced was not very significant.	raised and toothed areas were formed in concentric zone half way between the inoculum and the edge of the culture, and erect fruit bodies showing regular pore-tubes appeared here and there on this zone after an interval of eleven days from the date of inoculation. The pore tubes showed a well developed hymn-nlum consisting o f basidia with sterigmata and attached immature spores, mixed with a number of cystidia and tramal hyphae bearing clamps but no terminal spores.	inverted flask, very few being clavate. Thus, the fruit-formation was partially affected.
(*b*) No conidia were produced.		
(*c*) A few chlamydo-spores appeared after the last exposure but their number never increased.		

Damage in sub-cultures

A subculture was carried out in plate after the fifth exposure on 19th June, 1936. Smear-examinations from this subculture were identical in all respects to those from the main plate. Fruit-formation occurred after 10 days and was delayed by 3 days in comparison with the control. Pore-tubes were well-developed but they were all empty showing no trace of basidia or other organs and filled with dead tramal hyphae. The effects of radiation are, therefore, clearly evident in the subculture, though not to the same degree in the main plate, as there was total suppression of the development of reproductive organs.

On 25th June, 1936, a wood-block (mango-wood) subculture was carried out; growth on this wood-block culture was fair but it did not ultimately produce any fruit-body.

(2) A malt-agar plate was inoculated with *Trametes cingulata* on 9th June, 1936, and was exposed in the same manner to X-rays

at 150 K V., 3 m. a., at 30 cm. from the target for seven days for fifteen minutes on each day. Exposure was first given on 13th June, 1936, *i.e.*, 4 days after inoculation and ended on 20th June, 1936.

Damage in the main plate

The only effect produced was that living hyphae became dead and empty of cell-contents in large number. Such dead hyphae increased with increasing doses of X-rays. Living hyphae were all with clamps and with numerous mediate branches. Conidia became enormous with a good number of chlamydospores.

Recovery from damage in sub-cultures

Three subcultures were taken, *viz.*, after the first, third, and the seventh exposures. They showed no dead or damaged hyphae and were absolutely like the normal in every respect.

A mycelial transfer was made to sterilised wood-block (mango-wood) on 23rd June, 1936. Growth was rather stunted and checked at first, but later on the wood became completely covered up. No fruit-formation occurred in wood-block, in the irradiated plate or in any subculture.

The effect of X-ray exposure for *only fifteen minutes* on culture of *Trametes cingulata* at 150 K V. with 3 m. a. at 30 cm. distance from the target was studied. A malt-agar plate was inoculated with *Trametes cingulata* on 30th May, 1936. It was exposed to X-rays from a Coolidge tube only once on 6th June, 1936, (when the plate was full), *i.e.*, 7 days after inoculation, for a period of fifteen minutes only. The petri-dish lid was replaced by cellophane during exposure. The hyphae were all with clamps and with numerous mediate branches and conidia at the time of exposure and the culture showed distinct zonation. There were no chlamydospores.

The only effect produced by fifteen minutes' radiation was that a number of hyphae became dead, and many were damaged and lost clamps. Chlamydospores developed later as in the normal. Smear-examination from the zoned area showed numerous conidia and mediate branches while that from non-zoned area showed less conidia and less mediate branches. These features are also present in a normal culture showing zonation.

A mycelial transfer was made to sterilised wood-block (mango-wood) on 15th June, 1936. Moderately good growth was produced and smear-examination showed normal type of hyphae with numerous mediate branches, conidia and a few chlamydospores. No fruit-body appeared either in the irradiated plate or on the wood block.

Daily one hour X-ray (soft) exposure upon *Polystictus leoninus* for six days at 50 K V. 2 m. a.

A malt-agar plate-culture of *Polys. leoninus* inoculated on 13th November, 1936, was exposed to X-rays. The first exposure was given on 16th November, *i.e.*, 3 days after inoculation, and the last on 26th November, 1936. Altogether six exposures were given within this period and each exposure was of one hour's duration. The petri-dish lid was, as usual, replaced by cellophane. The potential and tube current employed in the case were 50 K V. and 2 m. a. respectively and the distance of the plate culture from the target was 18 cm. The hyphae at the start were all broad, with clamps and with a good number of mediate branches. There were no conidia, but a few chlamydospores were present and there was no trace of basidia when exposure was first begun. A separate plate-culture was kept as control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae Damage to the vegetative hyphae appeared first after the second exposure on 18th November, 1936, the first exposure having no effect. Hyphae became a little narrower than the normal and their protoplasmic continuity became broken by at places only. With increasing doses of radiation many more hyphae became more and more damaged and narrow. Vacuolation increased and the whole protoplasmic mass of living hyphae became broken up into disconnected chains. Some extremely narrow damaged hyphae without any clamps were produced. But their number always remained fewer and clamped hyphae whether broad or narrow, always preponderated over non-clamped ones. A good number of empty hyphae were produced and	Basidia first appeared in the irradiated plate on 20th November,'36, so that fruit body appeared after seven days. The control plate also fruited at the same time. But contrasted with the control, fruiting was very much affected, no regular and erect fruits developed on the culture. Some yellow porous areas were formed but these were distributed in patches over the culture and were not aggregated to form a complete circular zone round the inoculum. Smear-examination from these fruiting areas showed an immense number of dead hairs amongst which were found a few basidia The basidia were much smaller in size than those of the normal, were much shrunken in appearance and irregular in shape and were never in close cluster but irregularly	Basidia were found in good number when they first appeared, but with increasing exposures their numbers became much reduced. This was due to a large number of basidia undergoing disintegration and this was especially evident after the fifth exposure when a large number of disorganised basidia became aggregated in masses, took a deep stain and lost their distinct outline. No fruit-body was inserted in agar plate for spore discharge as no regular fruit could be found.

In the vegetative stage.	In the reproductive stage.	Observations.
these became double walled and were ultimately converted into hairs which increased in immensed number. (*b*) A few conidia were produced after an interval of twenty-one days after inoculation. (*c*) Chlamydospores entirely disappeared.	scattered, and t h e y were never found with sterigmata or attached spores.	

Changes in the first vegetative generation-Sub-cultures

Altogether five subcultures were carried out from the irradiated plate, the first after the first exposure, the second after the second exposure, the third after the fourth exposure, the fourth after the fourth and last exposure, and the fifth, 7 days after the last exposure. The first four subcultures were in plates while the last was in trube.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. While the first and the second subcultures showed hyphae which were all extremely narrow in comparison with the n o r m a l, with clamps extremely reduced in size wherever present, and with an abundance of s u c h hyphae rendered clampless by radiation and with protoplasm invariably broken up in disconnected c h a i n s, the third and the fourth subcultures s h o w e d hyphae of similar nature together w i t h hyphae almost resembling normal condition in about equal proportions; the fifth sub-culture s h o w e d an overwhelming preponderance of normal hyphae.	Fruit body appeared in all after eight days which falls a l m o s t within the n o r m a l period. The fruit-bodies were all very regular in a p p e a r a n c e, showing well-formed poretubes, and a fruit from each sub-culture was inserted in agar plate on 16th December 1936. No sporefall occurred from the fruits of the first four sub-cultues but spores were discharged from the fruit of the fifth subculture twice on 17th December, 1936 and 18th December 1936 though very scanty each time. Sections were taken from all and it was found that while the fruits of the first four sub-cultures had pore tubes filled only with dead trämal	In g e n e r a l, the damage caused in the main plate by radiation was found to persist in all but the degree of damage present varied to a considerable extent in each sub-culture. The first and the second sub-cultures showed damage to the same extent as that of the main plate, the third and the fourth sub-cultures s h o w. e d damage to a far lesser extent, while the fifth sub-culture s h o w e d only slight damage when compared with the normal.

In the vegetative stage.	In the reproductive stage.	Observations.
(b) Conidia whose presence in a non-conidial strain like *P. leoninus* is always associated with damage, appeared in all the subcultures within 6 to 8 days after inoculation. Their number was appreciably great in the first and second sub-cultures but became a few only in the third and fourth sub-cultures. The fifth sub-cultures whose condition was almost similar to the normal, had almost no conidia but a few chlamydospores. (c) No chlamydospores were found in the first four sub-cultures, a few chlamydospores were present in the fifth sub-culture.	hyphae, that of the last had a few living tramal hyphae provided with clamps but no terminal spores. Probably, the few basidia that shed spores had all been converted into tramal hyphae by the time the fruit of the last sub-culture had been sectioned. The spores were transferred to a malt-agar tube and kept inside an incubator at 37° C. Germination occurred after an interval of nine days from the date of transfer and clamps developed after eleven days. The tube was brought to light and a small yellow patch on the top of the plant suggesting a normal sporophore developed after sixteen days.	

Results in the second vegetative generation-Sub-cultures

The five subcultures of the first generation were transferred to fresh malt-agar tubes, the first and the second on 2nd December, 1936 and the rest on 5th December, 1936.

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. Smear-examination on 9th. December, 1936 showed that in every tube the majority of vegetative hyphae had reverted to the normal stage. Damaged hyphae showing broken up nature of protoplasm still persisted in small number but they	Fruit-body appeared in all the five tubes within the normal period after seven days. Basidia in close cluster, some even with sterigmata and attached spores, could be found on smear-examination. The fruit-bodies were all well developed showing regular poretubes and	Smear-examination showed absolutely normal hyphae of *P. leoninus* with clamps and mediate branches. Thus, complete recovery was obtained in the second vegetative generation.

In the vegetative stage.	In the reproductive stage.	Observations.
gradually disappeared from the cultures later on and normal healthy hyphae came into preponderance. (*b*) A small number of conidia developed in all but their number never increased. (*c*) Chlamydospores developed in all and they increased to good number, later on.	were inserted in agar plate on 21st December, 1936. No spore-fall occurred from the fruit of the first subculture (probably due to some defect in the mode of insertion) but spore-fall was obtained from all others on 23rd December, 1936 and continued for two days, though very scanty on each day. Sections showed that the poretubes contained basidia mixed with a few tramal hyphae bearing clamps but no terminal spores. But no attached basidiospores were found. The basidia were all of normal size and measured 26x10 μ. Spores from each of the four fruit bodies were then transferred to malt-agar tubes and kept inside an incubator at 37° C owing to the low temperature-conditions of the time. The spores germinated after five days and imperfect fruit-bodies appeared after twelve days (owing to being kept in the dark).	

Further sub-cultures

Mycelia from the above five tubes of the second vegetative generation were subcultured in malt-agar tubes upto the fifth generation but in no case was damage found to persist. Fruit-body appeared in every generation within the normal period and perfect and regular fruit-bodies were produced. The fruit-bodies were employed for various purposes when fresh spores were required, and in every case copious spore-fall was obtained from fruit-body of each tube. In every generation chlamydospores were totally absent when the culture was young and ultimately became numerous when the culture turned old. Conidia developed in very small number in every generation. They never increased in number and persisted right upto the end.

Growth in wood-blocks

Inocula from the main plate as well as from the first two sub-cultures of the first generation which showed the greatest amount of damage, were carried to sterilised wood-blocks (mango) on 30th November, 1936. Growth was moderately good in all. The wood-block inoculated from the main plate showed recovery on 22nd February, 1937, *i.e.*, after 85 days, while those inoculated from the two subcultures showed recovery after 50 days on 18th January, 1937. Fruit-body appeared in the wood-block subculture from the main plate on 13th April, 1937, *i.e.*, 104 days after inoculation and 19 days after recovery, while fruit-bodies in the other two wood-blocks occurred on 8th March, 1937, *i.e.*, 68 days after inoculation and 18 days after recovery. Control wood inoculated in April, fruited after 14 days. A small part of the sporophore from each of the three wood-blocks was taken and inserted in agar plates on 16th April, 1937. Spore-fall occurred from each of the two fruit-bodies of the wood-block subcultures from two first generation subcultures, though it was rather scanty. Spores germinated quickly inside a moist belljar. But the fruit-body from the wood-block subculture from the main plate did not shed any spores, though section showed that the pore tubes were densely lined with basidia measuring $26–30 \times 10\mu$ mixed with a good number of tramal hyphae bearing clamps only. No sterigmata or attached spores were found.

Recovery from damage

Damage appeared in the main plate of *Polystictus lconinus* after the second exposure on 18th November, 1936, and recovery of vegetative hyphae was noted in tube subcultures of the second vegetative generation on 9th December, 1936. The total period calculated from the date of damage was therefore 22 days.

Fruiting period of		Control fruited in	
(*a*) The Main plate	7 days	7 days	
(*b*) Sub-cultures :—			
(1) 1st. generation	8 days	6-8 days	
(2) 2nd. generation	7 days	7 days	
(3) 3rd. generation	10 days	10 days	winter months
(4) 4th. generation	11 days	11 days	
(5) 5th. generation	10 days	10 days	

Fruiting period in wood-blocks

	Total period	Period after recovery	Fruiting period in the control wood-block
Subcultures from the main plate	104 days	19 days	14 days
,, 1st. subculture	68 days	18 days	
,, 2nd. ,,	68 days	18 days	

16 hours' (soft) X-ray exposure upon *Polystictus leoninus* at 30 K V. 3-5 m.a.

A malt-agar plate-culture of *Polys. leoninus* inoculated on 5th February, 1937, was exposed to X-rays on 11th February, 1937, from a Heading tube which enabled exposures to be given for hours together without interruption. The potential was only 30 K V. and the tube-current varied from 3 to 5 m. a. By increasing the tube-current and lowering the potential, not only the output of X-rays increased but also the rays became very superficial—though much more penetrating than the ultra-violet rays. The lid of the petri-dish containing the culture was replaced by sterilised cellophane during the exposure, and the plate was fixed in front of one of the windows of the apparatus by clamps in such a manner that the rays fell vertically upon the culture, affecting an area of about 3 cm. in diameter round the inoculum. The distance of the culture from the target was kept at 10 cm. Exposures were given for five hours on 11th February, 1937, five hours on 12th February, and for six hours on 13th February, 1937, so that the total period of exposure was sixteen hours.

As the rays affected only a small area round the inoculum, two subcultures were carried in petri-dishes, immediately after the third day's exposure —one of this was from the irradiated region and the other from the non-irradiated region near the margin of the exposed plate. Another subculture was taken in malt-agar tube from the irradiated region six days after the last exposure. The irradiated plate and the subcultures were all kept in the diffused light of the room.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. Smears were taken from both the irradiated and non-irradiated	Fruit-body appeared in the main plate after thirteen days while the control fruited after nine days. Excepting	The damage that was produced by three exposures of total sixteen hours' duration was almost insignificant and

In the vegetative stage.	In the reproductive stage.	Observations.
regions of the culture. While smears from t h e n o n - irradiated region were absolutely like those from the normal, smear-examination from the irradiated region showed hyphae with prtoplasm slightly damaged and broken up at places only and clamps a little smaller than those in the normal. (*b*) Conidia developed eight days after inoculation and were found when the culture was first examined after the third exposure. But conidia were always a few in number and were restricted only to the irradiated portion. (*c*) Chlamydospores developed eight days after inoculation and were found when the culture was first examined after the third exposure, subsequently chlamydospores increased in large number and were found throughout the culture.	a delay of four days in the fruiting period no other effect was produced. Sporophore appeared normally in a circular zone round the inoculum and regular pores were formed. S m e a r - examination from these fruiting areas showed basidia in large number arranged in compact layer with sterigmata and attached spores, mixed with a large number of tramal hyphae bearing clamps but no distinct secondary spores. Basidia and spores were all healthy and agreed with the normal in measurements.	indistinguishable from the normal state.

Results in the sub-cultures

The two subcultures after the last exposure from the irradiated and non-irradiated portions in malt-agar plates fruited after twelve days, while the subculture in a malt-agar tube after an interval of six days from the last exposure fruited after nine days. Fruiting was, therefore, delayed by three days in the first two subcultures but occurred within the normal period in the case of the third subculture. On smear-examination, however, they were all found to be absolutely normal in the vegetative character of the hyphae and no damage was found in any of them. Fruit-bodies were very regular in all of them, showing basidia with attached spores, tramal hyphae, etc. Thus, except a retardation of three days in the appearance of fruit-bodies in the first two subcultures no other effect was produced.

Irradiation of spores by soft X-rays

Fresh spore-fall from a normal fruit-body of *Polystictus leoninus* in tube was obtained on two agar plates on 16th March, 1937. One plate was immediately exposed to soft X-rays at 50 K V., 2 m. a., from a Coolidge tube at a distance of 30 cm. from the target. Exposure was given for fifteen minutes and the petri-dish lid was replaced by sterilised cellophane during the exposure. Immediately after the exposure the irradiated plate as well as the other plate, which was kept as a control, were both kept inside an incubator at 37° C.

On 17th March, 1937, it was found that with the exception of a few spores all the irradiated spores had germinated. The control spores also germinated on 17th March, 1937. Subcultures were at once carried out in malt-agar tubes both from the control and irradiated spores. The tubes were kept inside incubator (in the dark). No growth occurred in any tube within five days, but on 23rd March 1937, further growth was noticed in all tubes containing irradiated as well as non-irradiated spores. Initiation of fruiting occurred in all tubes after seven days, but it took seven days more to develop tiny fruit-bodies showing regular pores as all the tubes were kept in the dark. Hyphae of subcultures obtained from X-rayed spores were similar in all respects to the normal ones, and no damaged hyphae were noticed. Two such fruit-bodies from irradiated spore-subcultures together with two control fruits were inserted in agar plates on 9th April, 1937. Copious spore-fall occurred from all the fruit-bodies within a few hours of insertion and continued for two days. Transfers of spores were again made to malt-agar tubes on 11th April, 1937, and fruiting occurred in all tubes within seven days. This time the tubes were kept in light. Sections showed preponderance of basidia in almost every pore-tube, bearing sterigmata and attached spores, mixed with a few tramal hyphae bearing clamps but no terminal spores. The basidia were 26–30×10–12μ and spores were hyaline, cylindrical and 10–12×5–6μ. This agrees exactly with the normal in measurements.

X-RAYS

Discussion and conclusion

The only work on effects of X-rays on Polypores so far attempted, I think, is that of Dickson (7). He irradiated young cultures of *Trametes serialis* and *Merulius lachrymans* in petri-dishes but could not get any positive result, the only change noticed was a slowing up of the growth rate due to the heating effect during irradiation. In the present case also only negative result was obtained, no saltant or mutant involving genic change was ever produced. With lower fungi (Mucor, Phycomyces and Chaetomium), however, Nadson and Philippov (12) and Dickson (7 & 8) obtained saltants which remained constant through a number of succeeding

generations. As with the ultra-violet radiation various degrees of injury were produced by X-rays, which have been recorded in the preceding pages. From the application of two kinds of X-rays— hard one of 150 K V. and soft rays of 50 K V. and 30 K V. it was found that small doses produced slight injuries from which there was quicker recovery, and that heavy doses totally suppressed sporophore-formation though conidia and chlamydospores (asexual spores) remained almost unaffected. Nadson and Philippov (loc. cit.) could destroy the formation of sexual organs (zygotes) of *Mucor* by strong doses of X-rays but they could never prevent the formation of asexual sporangia. Smith (16) also holds that "fungi are rather insensitive to X-rays but large doses produce killing effects." Killing effects are caused probably by toxic substances in the cytoplasm of cells exposed to the action of X-rays. In connexion with his study of disintegration of chromosomes at the first meiotic division in three species of *Orthoptera* by application of X-rays Whito (18) holds that their complete disintegration results from the destructive action of a substance produced or liberated in the cytoplasm as a result of irradiation.

Effects of radium-radiation on artificial cultures of three *Polypores* (*Polystictus leoninus, Trametes cingulata* and *Polypores ostreiformis*)

As in the case of X-rays both small and heavy doses were tried —5 mg. radium in the case of light dose and 120 mg. radium as heavy dose. The radium used was in the form of radium sulphate enclosed within small platinum capsules ·1 mm. thick, which transmit mostly Y-rays. In each case the radium was placed on the upper surface of the lid of the culture-dish at the centre. In a malt-agar plate irradiated with 15 mg. radium for half a minute and then inoculated with *Polyporus zonalis* and *Trametes cingulata* it was found that the growth was normal as in a non-irradiated plate.

Light dose of 5 mg. radium

(1) Two malt-agar plate-cultures of *Polystictus leoninus* inoculated on 21st July, 1936, were exposed to 5 mg. radium rays for one minute and half a minute on 24th July, *i.e.,* three days after inoculation. Subsequent smear-examination showed no damage to the vegetative hyphae and fruiting areas were formed in both the plates one day earlier than that in the control plate. Excepting a slight hastening of the reproductive period, the effect of such short exposures can neither be regarded as exerting any stimulating influence. Subcultures were carried up to the second vegetative generation, the growth was found to be perfectly normal and fruit-formation was regular as in the normal cultures.

(2) A malt-agar plate-culture of *Trametes cingulata* was subjected to 5 mg. radium rays for one minute on the eighth day from the inoculation date when the plate was completely full and showed distinct zonation in the culture. Hyphae were with clamps and a number of short mediate branches, it was a conidial strain full of

conidia, such highly conidial strains usually do not form fructifying areas. The plate was microscopically examined every day for more than a fortnight, but during the period absolutely no change was found. Subsequent subculture on sterilised wood-block showed normal vigorous hyphae with a number of conidia and chlamydospores.

(3) Another similar plate-culture of *Trametes cingulata* was similarly exposed to 5 mg. radium rays for five minutes. A good number of hyphae became damaged by radiation, the protoplasm of such hyphae became broken up into disconnected chains of oidia and many became altogether empty, and there was great increase in the number of conidia and chlamydospores. But subsequent transfers to sterilised wood-blocks showed almost complete recovery in the course of about three months.

(4) A half-grown malt-agar plate-culture of *Trametes cingulata* inoculated on 13th June, 1936, was exposed to 5 mg. radium rays for six hours on 17th June, 1936, *i.e.*, four days after inoculation. Smears were examined at every hour and a subculture was taken at the end of each hour of exposure.

Results in the main plate

The culture was all with clamped hyphae and with a large number of mediate branches, a few conidia and no chlamydospores were noticed before the exposure was begun. During the continuance of exposure a few dead and empty hyphae were produced and there was a progressive increase in the number of conidia at the end of each hour. No chlamydospores were produced. Smear-examination on the next day *i.e.*, 18th June, 1936, showed the presence of quite a good number of dead and empty hyphae. The protoplasm of living hyphae became broken up into fragments at many places and presented a damaged appearance. A slight increase in the number of mediate branches was noted. Conidia increased in large number and a good number of chlamydospores was produced.

Results in the plate sub-cultures

Altogether six sub-cultures were carried out on the 17th June 1936, one at the end of each hour's exposure. Complete recovery to the normal state occurred in all the subcultures on the 23rd June, 1936.

A transfer was made to sterilised wood-block (mango) on 23rd June, 1936. Smear-examination after a month showed a preponderance of conidia over chlamydospores. The majority of the hyphae were as broad as normal hyphae and were in the living condition, but a few dead hyphae also were present.

(5) A malt-agar plate-culture of *Polystictus leoninus* also was similarly exposed to 5 mg. radium rays for six hours continually on 17th June, 1936, after an interval of four days from the inoculation date. As in the case of *Trametes cingulata* smears were examined

at every hour and a subculture was taken at the end of each hour of exposure.

Results in the main plate

During the continuance of exposure no damage was caused to the vegetative hyphae, no conidia were produced, chlamydospores which were a few before the exposure was begun, remained the same. Subsequent examination of the irradiated plate did not show any damage to the hyphae, only the chlamydospores increased in fairly large number but there was never any conidia produced nor did the hyphae show any tendency to break up into oidial chains. Fruit-formation was not affected in any way, fruiting areas were formed within the normal period (seven days) and they showed regular and healthy basidia and attached normal spores.

Results in the sub-cultures

Examination of the six subcultures also showed no damage anywhere, erect fruiting areas were formed within the normal period and they were perfect with normal basidia in close clusters mixed with a few tramal hyphae clamped, and some of the basidia had sterigmata bearing mature spores.

Heavy dose of 120 mg. Radium

(1) A malt-agar plate-culture of *Polystictus leoninus* inoculated on 25th June, 1936, was exposed after four days, *i.e.*, on 29th June, 1936, for 48 hours continually to 120 mg. of radium. The petri-dish containing the culture was kept inverted during exposure and the radium was placed upon the upper surface of the bottom part of the petri-dish. The hyphae were all with clamps and with a good number of mediate branches. The culture was absolutely young, showing no conidia or chlamydospores or hairs and there was no trace of basidia when the exposure was begun. A separate plate-culture was kept as a control. Subcultures from the main plate were taken after the removal of the radium (after 48 hours).

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. Damage to the vegetative hyphae became evident on the very first smear-examination on 2nd July, 1936. The protoplasm of living hyphae became much vacuolated and broken up into fragments. As the culture became old, these damaged hyphae gradu-	Basidia first appeared in the main plate on 5th July, 1936 *i.e.*, 10 days after inoculation while the control fruited perfectly well after seven days. Fruit-formation was characterised by the appearance of toothed areas round the periphery of the culture. Smears from this area showed	The results of irradiation in the main plate are therefore in the direction of suppression of spore-formation, delayed and imperfect development of fruit-bodies and a gradual conversion of healthy and clamped hyphae into non-clamped damaged ones.

In the vegetative stage.	In the reproductive stage.	Observations.
ally increased in number and healthy hyphae almost disappeared. The protoplasm became more and more vacuolar and ultimately broke up into disconnected chains with a strong tendency to break up into oidia. Many of these hyphae altogether lost their cell contents and clamps and were ultimately converted into dead and double-walled hairs. Furthermore, the damaged hyphae became much narrower than the normal ones, clamps became extremely reduced in size, and the protoplasm presented a very much streaked appearance. But the number of clamped hyphae always preponderated over the non-clamped damaged hyphae. The number of dead hairs and empty hyphae gradually increased. (b) and (c) Conidia and Chlamydospores. · Conidia appeared almost simultaneously with chlamydospores on 27th July, 1936, i.e., 32 days after inoculation and both could be found in large numbers later on. But the number of chlamydospores was always greater than that of the conidia.	basidia in large number for the first few days. But these basidia never came to maturity. They were never found to bear any sterigmata or spores, were very much shrunken on the first appearance and were either irregularly scattered or sparsely clustered. A few living tramal hyphae could occasionally be found but no clamps or terminal spores were found in them. An erect fruit showing regular pores developed, however, on the culture on 10th July, 1936, i.e., after fifteen days, but on sectioning it was found that the pore-tubes were filled only with dead tramal hyphae and there was no trace of any living element. The basidia that were found in smears for the first few days gradually disintegrated later on.	

Damage in the sub-cultures (*i.e.*, first vegetative generation)

Two sub-cultures were carried out from the main plate, the first on 6th July, 1936, *i.e.*, 5 days after the removal of radium, and the second on 8th July, *i.e.*, 7 days after its removal.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Damage caused in the main plate by radiation was f u l l y transmitted to the sub-cultures. The vegetative hyphae were all extremely na r r o w e r than the normal with protoplasm broken up into disconnected c h a i n s of oidia. Healthy hyphae were altogether absent. A large number of such hyphae became non-clamped, emptied of all cell-contents and were converted into hairs. Clamps became e x t r e-mely reduced in size and the number of such damaged hyphae with extremely reduced clamps were almost equal to those which became non-clamped.	Fruiting areas as noted by the first appearance of basidia, appeared in the first sub-culture on 14th July, 1936, *i.e.*, eight days after inoculation and in the second sub-culture on 15th July, 1936, *i.e.*, seven days after inoculation. The f r u i t i n g p e r i o d for the control at that time was seven days. Toothed areas deve-loped round the peri-phery of the cultures and smear-examination f r o m these a r e a s showed a large number of basidia irregularly scattered and not in cluster. Basidia were much shrunken in ap-pearance and without a n y sterigmata o r spores. Erect fruit-bodies developed in both the plates, but on sectioning they did not show any basidia in the pore-tubes but a few living tramal hyphae bearing only clamps and no s e c o n d a r y spores.	No further transfers were made to any wood-block and the recovery process could not be studied owing to accidental contamina-tion of both the sub-cultures in the first generation.
(*b*) Conidia develop-ed in small number simultaneously w i t h chlamydospores. Coni-dia were first noticed in the first sub-culture en 23rd July, 1936, *i.e.*, seventeen days after inoculation, and in the second subculture on 20th June, 1936, *i.e.*, 12 days after inocula-tion.		
(*c*) Chalamydospores and conidia both in-c r e a s e d in large numbers later on but the number of conidia was found to be p r e p o n d e r a t i n g o v e r that of the chlamydospores.		

(2) A malt-agar plate-culture of *Polyporus ostreiformis* inocu-lated on 25th June, 1936, was subjected to 120 mg. radium on 29th June, 1936, *i.e.*, four days after inoculation when the plate was almost full. Radium was placed upon the upper glass surface of

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the lid of the petri-dish and removed after 48 hours on 1st July, 1936. The plate-culture showed microscopically all broad and clamped hyphae measuring $4-6\mu$ in breadth with a good number of mediate branches. The clamps were all broad measuring $3-4\mu$ in breadth. There were no conidia but a good number of chlamydospores were present. A separate plate-culture was kept as a control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. Damage to the vegetative hyphae was not very great. The first sign of damage was noted on 6th July, 1936, i.e., five days after removal of the radium. The protoplasm of some hyphae showed an increased vacuolation and became streaked in appearance or broke up at places only. A few dead and empty hyphae without any clamps were produced and these increased to good number later on. Excepting these changes no other effect was noticed in the vegetative phase. Healthy hyphae with normal clamps and mediate branches were always present in large number, and compared with these, the number of dead or damaged hyphae was insignificant. No narrowing of hyphae was noticed. (b) The main plate was examined for nearly two months but during this period no conidia had developed, nor was there any tendency of living hyphae to break up into oidia.	The reproductive phase, however, showed considerable damage. Fruiting areas appeared in the main plate on 25th July, 1936, i.e., after an interval of thirty days while the control fructified after twenty one days. A moist spot developed at one spot near the periphery of the main plate and in the course of four to five days it assumed a yellow colour. But no porous area was formed. A smear examination from this condensed yellow area showed the presence of a good number of basidia which were all rudimentary and without any sterigmata or spores, mixed with quite a good number of chlamydospores of varied shape and dimension.	Fruit-formation, therefore, was not only delayed by nine days, but the development of any regular fruit was totally suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
(c) The number of chlamydospores decreased a little as the culture became old and many empty chlamydospores could be found.		

Results in the sub-cultures (*i.e.* the first vegetative generation)

Two subcultures were taken from the main plate—the first on 6th July, 1936, *i.e.*, five days after the removal of radium and immediately after damage was noticed in the main plate, and the second on 8th July, 1936. *i.e.*, two days after the first subculture. These two subcultures, therefore, represent the first vegetative generation.

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. A little amount of damage in the form of hyphae with protoplasm broken up only at places was noticed for the first few days in both the sub-cultures. But later on, the damage totally disappeared and a smear-examination on 23rd July, 1936 showed complete recovery of the vegetative hyphae to the normal state. (b) No conidia developed. (c) Chlamydospores were present in moderately good number throughout.	Recovery was also noted in the reproductive phase. Moist spot developed on the wall of the plates in both the sub-cultures on 29th July, 1936, and on 31st July 1936 perfect porous areas were formed, so that fructification appeared in the first sub-culture after twenty-five days and in the second sub-culture after twenty-three days. The control fruited within twenty to twenty-five days. Fruiting areas were regular and sections showed basidia in close cluster bearing sterigmata and spores without any tramal hyphae elongations.	It should be noted that growth in the main plate stopped as soon as the hyphae reached the margin of the medium in the petridish. But in the sub-cultures growth was more vigorous. The hyphae not only ascended the walls of the plates but they could also overgrow the edges of the petridishes.

Recovery from damage

Damage was first noticed in the main plate on 6th July, 1936. Recovery was noted in both the plate subcultures (of the first vegetative generation) on 23rd July, 1936. The total period taken to recover was therefore 17 days.

Fruiting period of	Fruiting period in the control
(a) Main Plate	
(abortive fruit) .. 30 days	21 days
(b) Sub-culture:—	
(1) 1st. Sub-culture	
(regular fruit) .. 25 days	20-25 days
(2) 2nd. Sub-culture	
(regular fruit) .. 23 days	

(3) A malt-agar plate-culture of *Polystictus leoninus* inoculated on 18th September, 1936, was subjected to 120 mg. radium for six days continually from 23rd September to 29th September, 1936. The radium was applied to the glass surface of the bottom part of the petri-dish containing the culture, which was kept inverted during the exposure. The hyphae were all with clamps and with a good number of short mediate branches arising at right angles from the main hyphae, were non-conidial with a few chlamydospores and did not show any trace of basidia or dead hairs when the exposure was started. A separate plate-culture was kept as a control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae	Basidia were first noted in the main plate on 29th September, 1936, so that rudimentary fruit-formation took place in the course of eleven days after inoculation. The control fruited perfectly well after six days. The fruiting area was not developed in a circular zone but formed in patches of toothed areas round the peri-	Not only the vegetative phase but the reproductive phase as well was extremely affected by such long exposure to heavy dose of radium. No erect fruit-bodies ever developed on the culture-plate.
Damage to the vegetative hyphae became evident on the very first smear-examination on 26th September 1936, i e. after three days' exposure. The protoplasm of many vegetatative hyphae became much vacuolar and broke up at places and there was absolutely no trace		

In the vegetative stage.	In the reproductive stage.	Observations.
of basidia. After six days' exposure damage became more pronounced. Healthy hyphae almost disappeared and every hyphae showed the damaged character. T h e hyphae became much narrower than the normal ones, much more vacuolated, and broke up at places. As the culture grew old this broken up nature of the hyphae became more prominent and a strong tendency to break up into oidia was evident. A large number of healthy h y p h a e totally lost their clamps and cell-contents, and became converted into dead and double-walled h a i r s. The clamps became extremely reduced in size a n d m a n y empty h y p h a e with such clamps, either wholly or partially empty but still attached, could be s e e n. But clamped h y p h a e always preponderated over such non-clamped h y p h a e. Extreme narrowness o f t h e vegetative h y p h a e whether clamped or non-c l a m p e d was a noticeable feature. (*b*) A few conidia developed on 19th October, 1936, *i.e.* nearly a month after inoculation, but instead of increasing in number they altogether vanished. (*c*) Few chlamydospores that were found in the beginning disappeared entirely. No conidia or chlamydospores could, thus, be found in the main plate at the end.	phery of the culture, easily distinguished from the vegetative a r e a by their yellow colour. The pores were v e r y s h a l l o w and s m e a r - examination from t h e s e a r e a s showed a good number of basidia which never came to maturity. The basidia were very much smaller than the normal ones and shrunken in appearance, they were never in cluster, but always very irregularly s c a t t e r e d without showing anywhere any trace of sterigmata or spores. No tramal hyphae could be found amongst them. T h e basidia were present in good number at f i r s t but gradually t h e y underwent disintegration.	

Damage in the plate sub-cultures (first Vegetative generation)

Altogether five subcultures were carried out from the main plate—the first on 26th September, 1936, after three days' radium exposure, the second on 29th September, 1936, after six days' radium exposure, the third on 5th October, 1936, six days after the removal of radium, the fourth on 26th October, 1936, twenty-seven days after the removal of radium and the fifth on 29th October, 1936, *i.e.,* thirty days after the removal of radium. The first four subcultures were in malt-agar plates and the last in malt-agar tube. These five subcultures may be broadly divided into two groups (I & II) so far as their characters are concerned, the first two comprising one group and the last three comprising another group.

In the vegetative stage.	In the reproductive stage.	Observations.
I. (*a*) Hyphae Damage caused by radiation upon the plate was fully transmitted to all the sub-cultures of the first generation. But the extent of damage varied in the two groups of sub-cultures. In the first and second sub-cultures comprising the first group, the vegetative hyphae were all damaged with protoplasm broken up into fragments, they were much shrunken and much narrower than the normal ones at places only. Nevertheless, a large number of hyphae could be found which though damaged, were as broad as the normal ones, but with protoplasm very much streaked in appearance. Hyphae were all damaged, but still clamped hyphae preponderated over the non-clamped hyphae. (*b*) A tendency to break up into oidia was not much apparent but a small number of	Fruit formation (as noted by the first appearance of basidia) occurred in the first and second sub-cultures after ten days, and in the third, fourth and fifth sub-cultures after twelve days. The fruiting period in the control varied at that time from six to seven days. As regards the reproductive phase, some differences could be noticed between the two groups of sub-cultures. In the first and second-sub-cultures a good number of basidia could be found arranged in rather sparse cluster, so that a tendency to form a hymenial layer was evident. No basidiospores were found. Erect fruits developed in both and were inserted in agar plates on 12th December, 1936. But no spore-fall occurred, and sections showed that their pores were filled with dead tramal hyphae and there was no trace of any basidia or living element.	It may be stated in general that damage to both the vegetative and reproductive phases was far greater (and almost to the same extent as in the main plate) in the case of the last three sub-cultures than in the case of the first and the second.

In the vegetative stage.	In the reproductive stage.	Observations.
c o n i d i a developed nearly a month after inoculation i n b o t h. the plates. Their number never increased but they rather f i n a l l y vanished. (*c*) Chlamydospores developed in s m a l l number at first but increased to immense number later on. II. (*a*) Hyphae In the third, fourth and fifth sub-cultures, the conditions were rather different. Damage to the vegetative hyphae was rather more pronounced and no wider hyphae could be found as in the two previous sub-cultures. H y p h a e were all extremely narrower than the normal ones and the sizes of the clamps were extremely small. The majority of the hyphae had no clamps, so that non-clamped hyphae were in preponderance over clamped hyphae. (*b*) A tendency to break up into oidia was more marked and, as a matter of fact, conidia appeared in all in the course of thirteen to fifteen d a y s a f t e r inoculation, which is far more quicker than that in the previous case. (*c*) Chlamydospores developed in all, but contrary to the cases of the first and second sub-cultures the number of conidia went on increasing in immense number while the number of chlamydospores became reduced to a few only.	In the third, fourth and fifth sub-cultures toothed areas were formed but no erect fruit-bodies ever developed. Basidia could be found in smears only from the fruiting areas and even then, they were only a few in number, very irregularly scattered and very much shrunken in appearance. No sterigmata or spores were found.	

Results in the tube sub-cultures (second vegetative generation)

As the third, fourth and fifth subcultures of the first generation showed a decidedly greater amount of damage than the rest, they were again subcultured in malt-agar tubes on 2nd December, 1936, to find out how far the damage persisted.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In this generation a partial recovery of the vegetative hyphae was noted. Hyphae were, on the whole, a little narrower than the normal ones and with clamps a little narrower in size, but the fragmented nature of the protoplasm was evident at some places only and not everywhere. Wider hyphae with protoplasm much vacuolated or streaked in appearance together with narrow hyphae filled up with protoplasm could be found in almost equal number with the damaged hyphae. Clamped hyphae came into preponderance and such clamped hyphae which were densely filled up with protoplasm, did not show any damage except for the fact that they were narrower than the normal ones. A good number of damaged hyphae became converted into hairs at the approach of the fructifying period. (*b*) A few conidia developed in all, though after seven days from the inoculation-date, but their number never increased. (*c*) Chlamydospores developed in small number.	Fruiting areas developed in all three sub-cultures of the second generation on 9th December, 1936, *i.e.*, seven days after inoculation, which falls within the normal period. The fruiting area was confined to the top of the slant in the form of a condensed yellow spot which afterwards formed pore-tubes. Smear-examination from these fruiting areas showed the presence of quite a good number of basidia arranged - either in sparse cluster or irregularly scattered. But never were any sterigmata or spores found. However, three fruit-bodies from three tube sub-cultures were taken and inserted on the lid of an agar plate on 25th December, 1936. Absolutely no spore-fall occurred even after three days under moist bell-jar, and a section on 28th December, showed that the pore-tubes had a few rudimentary basidia here and there without any spore or living tramal hyphae.	It is thus evident that though there was a partial recovery of the vegetative phase in the second generation, there was no sign of recovery in the reproductive phase.

Results in the tube sub-cultures (third vegetative generation.)

As only partial recovery was noted in the second generation, the third, fourth and fifth subcultures were again subcultured in malt-agar tubes on 25th December, 1936.

In the vegetative stage.	In the reproductive stage.	Observations.
(a) Hyphae. Smear examination on 31st December, 1936 showed that the vegetative hyphae had completely reverted to the normal state. A few damaged hyphae could still be found in the third subculture but they resembled those that are found in old normal cultures. However, this damaged character did not persist long. Complete recovery of the vegetative hyphae was noted in all. The hyphæ became as broad as the normal ones and were filled up with protoplasm without showing any streaked, broken up or vacuolated character. A good number of vegetative hyphae became converted into hairs with the approach of the fruiting period. (b) Conidia altogether disappeared. (c) A small number of chlamydospores developed and they gradually increased in number. Increase of chlamydospores takes place also in normal cultures when they become old.	The first appearance of basidia was noted in smear examination on 4th January, 1937 in all, so that fruit-formation was noticed in all after ten days. A fruiting period in the control at that time was also ten days due to reduced room-temperature in winter. The basidia were arranged in sparse cluster and were never with any sterigmata or spores. Fruit-bodies showing regular pores developed on the tops of slants in all and three fruit-bodies of three subcultures were inserted on the lid of an agar plate on 9th January, 1937. No spore-fall occurred from any of them even after six days under a moist bell-jar, and a section on 15th January, showed that the pore-tubes had only a few undeveloped basidia mixed with a few tramal hyphae without clamps or spores. A peculiar feature was that a large number of chlamydospores was found within each poretube.	It is therefore seen that damage to the reproductive phase still persisted to a great extent in the third generation, although complete recovery in the vegetative phase was noted.

Recovery in the fourth vegetative generation

The third, fourth and fifth subcultures were again subcultured in malt-agar tubes on 7th January, 1937. The vegetative hyphae

did not show any damage. They were normal in all respects showing a good number of mediate branches. No conidia developed, but a few chlamydospores were found.

Fruit-formation was noted in all after eleven days on 18th January, 1937, the control also fruiting at the same time. The fruit-bodies formed were very regular, showing well-developed pore-tubes, and three fruit-bodies from three subcultures were inserted on the lid of an agar plate on 25th January, 1937. Spore-fall occurred within four hours of insertion and continued for three days upto 27th January, 1937. Sections showed pore-tubes densely lined with basidia bearing sterigmata and spores (agreeing with the normal ones in measurements) mixed with a few tramal hyphae bearing clamps and some tramal hyphae bearing terminal globular spores. Spores dropped from these three fruit-bodies of three subcultures were transferred to three malt-agar tubes on 27th January, 1937. Growth was vigorous in all of them and fruit-bodies appeared in them within ten days after the transfer.

Results in the tube sub-cultures (fifth vegetative generation)

Subcultures were carried for one generation more in malt-agar tubes. In no case was any damage noted either in the vegetative phase or in the reproductive phase and the cultures were normal in all respects. No conidia developed, though a few chlamydospores were found. Regular fruit-bodies appeared after ten days, which falls within the normal period.

Transfer to wood-blocks

Out of the five subcultures of the first vegetative generation from the main plate, the last three, viz., the third, fourth and fifth subcultures were transferred to wood-blocks (mango-wood) on 30th November, 1936. The wood-block cultures, therefore, represented the second vegetative generation. The damaged nature of the hyphae persisted in all for more than three months, but ultimately all of them showed recovery and formed regular fruit-bodies. Recovery was noted in the third and fourth subcultures in wood-blocks on 15th March, 1937, i.e., after 105 days, and in the fifth subculture in wood on 5th March, 1937, i.e., after 95 days. It was, thus, a case of complete recovery. Conidia which developed in small number in all wood-blocks in the beginning, were later on entirely replaced by chlamydospores.

Small fruit-bodies appeared in the third subculture on 1st April, 1937, i.e., 121 days after inoculation and 16 days after its recovery, in the fourth subculture on 27th March, 1937, i.e., 117 days after inoculation and 12 days after its recovery; in the fifth subculture on 20th March, 1937, i.e., 110 days after inoculation and 15 days after its recovery. The fruiting period for the control

in wood-block culture at the time was 14 days. The fruit-bodies were well formed showing regular pore-tubes, and three such fruit-bodies from three wood-block subcultures were inserted on the lid of agar plates on 16th April, 1937. Copious spore-fall occurred on 17th April, 1937, but it became less on 18th April, 1937, after which it gradually stopped. Sections on 19th April, 1937, showed pore-tubes lined with basidia some bearing sterigmata and spores mixed with a few tramal hyphae bearing clamps but no terminal spores. Spores were not transferred to any malt-agar tube, but the agar plates containing discharged spores were kept inside a moist belljar. Clamps developed after five days and smear-examination showed normal hyphae of *Polystictus leoninus* with mediate branches and chlamydospores.

Recovery from damage

Damage was noticed in the main plate on 26th September, 1936, after the plate had received a continuous 120 mg. radium exposure for three days. The damage persisted right through the first vegetative generation in malt-agar plates. Partial recovery of the vegetative hyphae occurred in the second vegetative generation in malt-agar tubes. Complete recovery of the vegetative hyphae was noted in third generation in malt-agar tubes on 31st December, 1936, *i.e.*, after 96 days, though the damage still persisted in the reproductive stage. The period for recovery in wood-block subcultures, as has been said, varied from 95 to 105 days.

Summary of results

	Fruiting period.		Fruiting period in the control.
No Regular fruit-bodies formed	(A) In Main Plate	11 days	6 days
	(B) In Sub-cultures :—		
	I. 1st. vegetative generation	10-12 days	6-7 days
	II. 2nd. „ „	7 „	7 days
	III. 3rd. „ „	10 „	10 days
Regular fruit-bodies formed	IV. 4th. „ „	11 „	11 days
	V. 5th. „ „	10 „	10 days

Recovery and Fruiting period in wood-block sub-cultures

(Fruiting period in the control wood-block culture —14 days)

Wood-block culture from	Inoculated on	Recovery on	Period of recovery from damage	Fruiting on	The whole fruiting period	Fruiting period calculated from the date of recovery
3rd. sub-culture	30-11-36	15-3-37	105 days	1-4-37	121 days	16 days
4th. sub-culture	30-11-36	15-3-37	105 days	27-3-37	117 days	12 days
5th. sub-culture	30-11-36	5-3-37	95 days	20-3-37	110 days	15 days

50 mg. Radium-exposure upon *Trametes cingulata* in culture for ten days

A full grown malt-agar plate-culture of *Trametes cingulata* inoculated on 9th April, 1936, was exposed to 50 mg. radium on 25th April, 1936, *i.e.*, after an interval of sixteen days from the inoculation date. The radium was this time placed direct upon the surface of the culture by removing the lid, and the radium capsules were removed from the culture on 5th May, 1936, after a continuous exposure of ten days. The hyphae were all with clamps and with a large number of short mediate branches and there were a large number of conidia and a small number of chlamydospores before the exposure was begun. A few empty hyphae were present and the distinction between zoned and non-zoned areas altogether disappeared.

Damage in the main plate

Smear-examinations were carried out daily both during the exposure and after the exposure had been stopped. With increasing exposures a large number of hyphae became damaged so that a large number of dead hyphae were produced. The protoplasm of

such hyphae became very much broken up into disconnected chains and a strong tendency to break up into oidia became more and more evident in the living hyphae. Clamps became extremely reduced in size wherever present, and the majority of the hyphae became extremely narrower than the normal ones and non-clamped. Conidia gradually increased in immense number and chlamydospores disintegrated in large number and almost disappeared. A large number of empty chlamydospores was found. The culture was not totally killed but dead hyphae ultimately preponderated over the living ones, all of which became distantly branched and showed the damaged nature distinctly.

Damage in plate sub-cultures (first vegetative generation)

Altogether five subcultures were carried out from the main plate during ten days of radium-exposure. The first subculture was after two days' exposure, the second after four days' exposure, the third after six days' exposure, the fourth after nine days' exposure and the fifth and the last after ten days' exposure.

Damage noticed in the main plate was transmitted to all the subcultures but the extent of damage was far less. Damaged hyphae with broken up protoplasm or totally empty and dead hyphae were present in all in fairly large number but living hyphae preponderated in all. Moreover, the number of clamped hyphae was far greater than the non-clamped hyphae. Conidia were present in all together with a small number of chlamydospores.

The third, fourth and fifth subcultures were transferred to a dark room on 13th May, 1936, and kept in the dark thereafter. By keeping these three subcultures in the dark there was a sharp decline in the number of conidia, most of which developed a thick wall round them and became converted into chlamydospores. The number of chlamydospores so immensely increased that conidia almost vanished, while in the first and second subcultures kept in diffused light there was no reduction in the number of conidia and a few chlamydospores always remained. But such conversion into chlamydospores also takes place in the control kept in the dark, especially as the culture becomes old.

Transfer to wood-blocks (second vegetative generation)

Inocula from the third, fourth and fifth subcultures were transferred to sterilised wood-blocks (mango-wood) on 11th June, 1936. Examined on 8th August, 1936, they showed an equal proportion of damaged and healthy hyphae but the immense number of chlamydospores present in the plate subcultures of the first vegetative generation was entirely replaced by almost the same number of conidia.

No fruit-formation was noticed anywhere either in plates or in wood-blocks as it was a conidial non-fruiting strain.

Irradiation of freshly shed spores of *Polystictus leoninus* by 20 mg. radium for one hour

Fresh normal spores discharged from an artificial fruit-body of *Polystictus leoninus* were caught on two agar plates on 18th March, 1937. One plate was immediately exposed to 20 mg. radium, which was placed on the back of the plate so as to be nearer to the spores. The other plate containing spores was kept as a control. Irradiation was continued for an hour, after which the irradiated plate and the control-plate were both kept under a moist belljar in the culture room. On the next day, *i.e.*, 19th March, 1937, it was found that the irradiated as well as the control spores had all germinated. The irradiated spores were at once transferred to two malt-agar tubes and the spores from the control plate to another malt-agar tube. The tubes were kept in the diffused light of the culture room.

For two days no growth was noticed in any of the tubes. Growth was found to start in all on 22nd March, 1937, *i.e.*, on the third day after the transfer of spores. Linear growth was equal in all but the culture obtained from radiated spores was more fluffy than that from the normal spores. This condition persisted for the first few days after which growth in all three tubes became equal in nature. Fruit-formation appeared in the radium exposed tube-sub-cultures after twelve days and in the control-tube after thirteen days. The fruit-bodies were all regular showing well-developed pore-tubes, they were all (including the control-fruit) inserted in agar plates on 3rd April, 1937. The spore-fall was copious from all three fruits and occurred within three hours of insertion and continued for four days though showing a gradual decrease. The agar plates containing the discharged spores were kept under a moist belljar. Germination was quick and normal in all and clamps developed after five days in agar plates.

Mycelia from the three tubes just mentioned, of which the fruit-bodies had shed spores, were subcultured again on 31st March, 1937, in malt-agar tubes. The linear growth was the same and equally vigorous in all. Fruit-bodies appeared in all on 7th April, 1937, *i.e.*, after seven days and were very regular. Three fruits, one from each tube, were inserted again on 9th April, 1937, in agar plates for spore-fall; one of the fruit-bodies, as before, belonged to the control. Copious spore-fall occurred on the same day from all of them within three to four hours of insertion and continued for four days. No spore-fall occurred, however, during the day-time but only at nights. The agar plates containing the spores were kept under a moist belljar and all the spores germinated quickly. No further transfers of spores were carried out.

Smear-examinations, whether from the control-culture or from those obtained from germination of irradiated spores, did not show any difference, and all of them were quite normal in character.

RADIUM RAYS
Discussion and conclusions

It has been recorded by previous workers that strong doses of gamma-rays usually produce harmful effects on fungi and that especially heavy doses are necessary to produce lethal action. In the case of three Polypores treated with heavy doses of radium we had retardation of the vegetative growth, damage of the vegetative hyphae, suppression of spore-formation and delayed and imperfect development of fruit-bodies as described in the preceding pages; in some cases there was an increase in the number of chlamydospores. Ultimately, in subcultures (succeeding vegetative generations) recovery was noted within a varying period in all even where the radium was placed direct on the hyphae continuously for ten days, but it was usually found that recovery in the vegetative phase was quicker than in the reproductive one. Polypores, thus, seem to be extremely resistant to radium; Dauphin (6) exposing lower fungi to radium rays obtained sudden cessation of mycelial growth and of germination of spores but they were not killed because they began to grow again when brought to their normal condition. In the case of light doses of 5 mg. radium the damage was slight and recovery was very quick. Germination of spores was not affected in any way by exposing freshly shed spores of *Polystictus leoninus* to 20 mg. radium for one hour. Here no permanent change in the form of saltation or mutation could be produced; Lee, Haines and Coulson (11) could obtain besides lethal action only temporary inhibition of cell-division by exposing bacteria to 920 mg. of radium, they divided normally when they were removed from the radium rays and transplanted to fresh medium. Sibilia (15) has reported, however, two saltants from *Heterosporium gracile* by the action of radium rays, which have remained unchanged through several successive generations, though he was quite unsuccessful with ultra-violet radiation. It has been found that irradiation of the malt-agar medium with 15 mg. radium for half a minute had no toxic effect on growth of Polypores on it. Our experimental results show that *Polystictus leoninus* in majority of cases is more sensitive to radiation (sunlight, ultra-violet, X-rays and radium) than *Polyporus ostreiformis* or *Trametes cingulata*. These Polypores, I have found, usually fruit only in light, in complete darkness either they do not fruit at all or form in a few cases very imperfect and abnormal fruiting areas.

Literature Cited

1. BARNES, B.—Induced variation. Trans. Brit. Mycol. Soc. Vol. 20. 1935.

2. BOSE, S. R.—Sexuality of *Polyporus ostreiformis* and *Polystictus hirsutus*. La Cellule, Tome XLII, Fasc. 3. 1934.

3. BRODIE, H. J.—The Occurrence and Function of Oidia in Hymenomycetes. Amer. Journ. Bot. Vol. 23, No. 5. May, 1936.

4. BURKHOLDER, P. R.—The rôle of light in the life of plants. Botanical Review, Vol. 2, No. 1. 1936.

5. CATCHESIDE, D. G.—Biological effects of irradiation. Scientific Journal of the Royal College of Science Vol. VI. 1936.

6. DAUPHIN, J.—Influence of Radium on the development and growth of the lower fungi. Nature, Vol. 69. 1904.

7. DICKSON, H.—The effect of X-rays, Ultra-violet light and Heat in producing saltants in *Chaetomium cochliodes* and other Fungi. Ann. Bot. Vol. 46. 1932.

8. DICKSON, H.—Saltation induced by X-rays in seven species of *Chaetomium*. Ann. Bot. Vol. 47. 1933.

9. DILLON-WESTON, W. A. R. & HALNAN, E. T.—The Fungicidal action of Ultra-violet Radiation. Phytopathology, Vol. 20. 1930.

10. FAILLA, G.—Ionization and its Bearing on the Biological effects of Radiation. Chap. III of Duggar's Biological Effects of Radiation, Vol. 1. 1936. McGraw Hill Publication.

11. LEA, D. E., HAINES, R. B. and COULSON, C. A.—Action of Radiation on Bacteria. Proc. Roy. Soc. (B, Vol. 123. 1937.

12. NADSON, G. A. and PHILIPPOV, G. S.—Influence des rayons X sur la sexualité et la formation des mutantes chez les champignons inférieures (Mucorinées). C. R. Soc. Biol. Tome XCIII. 1925.

13. RAMSEY, G. B. and BAILEY, A. A.—Effect of ultra-violet radiation upon sporulation in *Macrosporium* and *Fusarium*. Bot. Gaz. Vol. 89. 1936.

14. SAX, KARL.—Effect of variations in temperature on nuclear and cell-division in *Tradescantia*. Amer. Journ. Bot., Vol. 24, No. 4. April, 1937, p. 223.

15. SIBILIA, C.—Saltazioni in Heterosporium gracile. Estratto del. Bolletino della R. stazione di Patologia V Vegetale di Roma, Anno XIV, N. S. 1934.

16. SMITH, E. C.—Effect of Radiation on Fungi. Chap. XXVII of Biological effects of Radiation (by Duggar), Vol. II. 1936. McGraw Hill Publication.

17. STEVENS, F. L.—Effects of ultra-violet radiation on various fungi. Bot. Gaz. Vol. 86. 1928.

18. WHITE, M. J. D.—The effect of X-rays on the First Meiotic Division in three species of Orthoptera. Proc. Roy. Soc. (B) Vol. 124. Nov. 1937.

STUDIES ON CAPPARIDACEAE—III
GENUS CAPPARIS

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Introduction

In the two earlier papers (5, 6), the writer had dealt with the life histories of *Maerua arenaria* and *Gynandropsis pentaphylla.* In the present paper are dealt with, three species of the genus *Capparis—C. galeata, C. horrida,* and *C. sepiaria.* The genus *Capparis* belongs to the same tribe as *Maerua,* namely, Cappareae, while *Gynandropsis* and *Cleome* belong to quite another tribe, Cleomae (3, 4). In a previous paper (5) a comparison was instituted between *Maerua* and *Gynandropsis;* in the present paper, in the light of the observations on the species of *Capparis,* will be discussed the justification of the above classification of the Capparidaceae, which was based on external morphology.

A general review of the literature concerning Capparidaceae has already been given in my previous papers. As for the literature upon the genus *Capparis,* Guignard (1) has studied the structure of the integuments in some species besides *Polanisia graveolens* and some *Cleome* species. Schiller (7) has done some purely cytological work upon *Capparis.* The paper of Mauritzon (4) deals, though

rather incompletely, with the megasporogenesis, among other genera
in *C. frondosa,* and *C. rupestris.* Regarding the species of
Capparis with which this paper deals, I have found no literature
except a very short account by Hedayatullah (2) upon *C. horrida*
in the proceedings of the Indian Science Congress.

Material and Methods

Fixed material of *C. sepiaria,* and *C. horrida,* was kindly given
by Prof. N. K. Tiwary, and that of *C. galeata* by Prof. A. C. Joshi.
Floral stages of *C. sepiaria* and *C. horrida* were fixed in Allen's
modification of Bouin's fluid, and the other species in formalin
acetic alcohol. The usual paraffin method was employed. Staining
was done mostly with Haidenhain's iron-alum haematoxylin. A
combination of safranin and gentian violet was also used.

Capparis galeata

The ovule arises as usual as a short cylindrical outgrowth
(Fig. 1). The two integuments arise almost simultaneously upon
this, but in the younger stages, usually, the outer grows faster than
the inner (Figs. 2 and 3). By the time the megaspore mother cell
is organised (Fig. 3) they envelop the greater portion of the
nucellus. At the base of the nucellus is found a growth to one side,
indicated by a bulge (*a*, Fig. 3). The continuance of this one-
sided growth results in the condition of the ovule shown in Fig. 4.
The embryo-sac at this stage is fully mature. The funiculus is
attached, consequent on the above-mentioned growth, not at the
base of the nucellus, but to one side. The adult ovule is campy-
lotropous in form (Fig. 5), and this form is attained by the simple
curvature of the ovule on either side of the point of attachment of
the funiculus. Thus, the funiculus is not attached throughout the
greater part of its length, but only at the terminal point. This is in
contrast to the condition in *Gynandropsis* and resembles that in the
two species of *Capparis* investigated by Mauritzon.

The ovary is septate from the earliest stage onwards (Fig. 6),
the septa arising as extensions of the placental regions, loosely lying
together, and enclosing a hollow canal in the centre. In old stages
also, this condition is preserved (some time the septa do not extend
quite up to the centre of the ovary in older stages due to their
inability to keep pace in growth with the increase in bulk of the
ovary). But usually the ovary is completely septate (Fig. 7), the
united portions of the septa in the centre giving roughly the appear-
ance of a central column of tissue, with a stylar canal. In these
older stages, some of the ovules are seen to be arising on the septa
(Fig. 7) and this seems to be due to the ingrowth of septal tissue
from the placental region.

In some cases, however, the central hollow space enclosed by
the septa is obliterated by their very loose parenchymatous tissue.

The primary archesporial cell is hypodermal in position (Fig. 8) and this cuts off very soon after its differentiation, a primary parietal cell (Fig. 2). Further periclinal divisions in this latter result in the sporogenous cell being sunk about 5 or 6 cells deep in the nucellus (Fig. 3). The sporogenous cell enlarges in size, and forms a linear tetrad of spores, of which the lowermost functions and the other three degenerate (Fig. 9). The functioning megaspore develops into the normal embryo-sac (Fig. 10) as usual. The young 8-nucleate sac is about 3 or 4 times as long as it is broad, the micropylar end being broader than the chalazal. In the young stages, it is often pointed at the latter end. As usual, egg apparatus at the micropylar end and the three antipodals at the chalazal end are organised.

The egg cell (Figs. 11 and 12) lies usually at the base of the synergids but sometimes at the same level. It is either rounded or triangular in section and in the older stages, bears a vacuole on the micropylar side of the nucleus. Its cytoplasm is less dense than in the synergids.

The synergids are usually flask shaped (Fig. 11) and are capped for a short distance, by a filiform apparatus. The nucleus lies in the centre of the synergid. In some embryo-sacs, a very slight beak like protrusion is noticeable beneath the filiform apparatus.

The antipodal cells are wedge-shaped and they completely degenerate early. In some ovules the wedge-shaped antipodals are slightly elongated towards the chalazal side (Fig. 13).

The polar nuclei are almost of the same size as the others. The secondary nucleus (Fig. 12) is formed in the ordinary manner and is situated a little away from the egg cell. At about the time of fertilisation, the synergids completely degenerate. The next available stage contains the fertilised egg with the remains of the degenerating synergids and numerous endosperm nuclei in dense cytoplasm (Fig. 14).

The pro-embryo consists of a linear row of 7 or 8 cells, the apical three cells of which are at first divided by longitudinal walls and a bunch of cells (Fig. 15) is formed. Some of the cells of the suspensor also usually divide once longitudinally (Fig. 16) and divisions in the suspensor cells nearest to the embryonal mass also contribute towards the formation of the embryonal sphere. The latest available stage (Fig. 17) shows a relatively short suspensor, and an embryonal sphere in which there is as yet no sign of any differentiation. The ovary at this stage is nearly 1¾ inches in diameter. In the micropylar part of the embryo-sac is dense cytoplasm in which are embedded numerous endosperm nuclei. At the chalazal end of the embryo-sac is a very small group of endosperm nuclei, and a faint peripheral layer of cytoplasm connects the two groups. Cell formation in endosperm is not found.
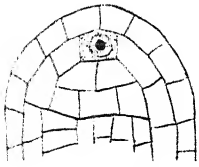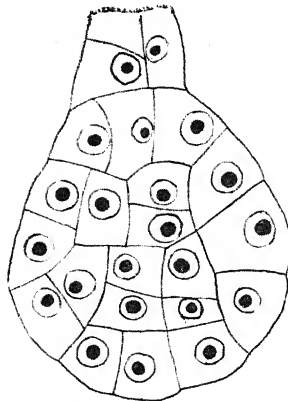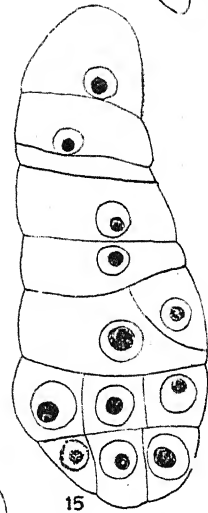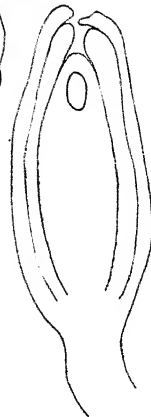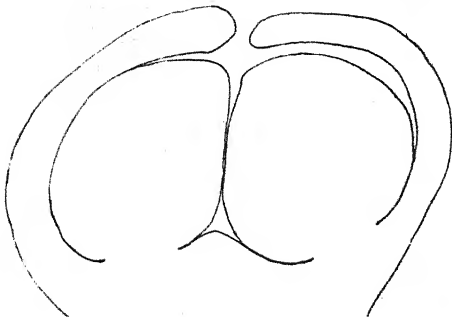
Degenerations.—Degenerations in the embryo-sacs are very common and are extensive from the uninucleate stage of the embryo-sac onwards. It is in comparatively very few ovules that embryos are developed. Degenerations are most common in the mature embryo-sac. In Fig. 11, one of the polar nuclei is in a condition of hypertrophy. Even some embryos undergoing complete degeneration are found.

Starch grains are numerous in the embryo-sacs.

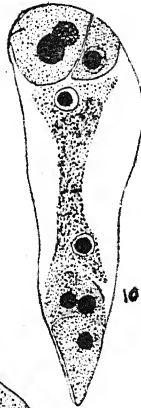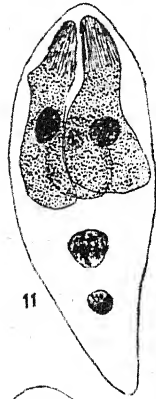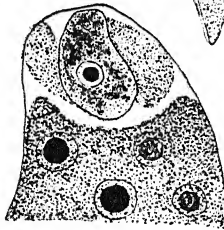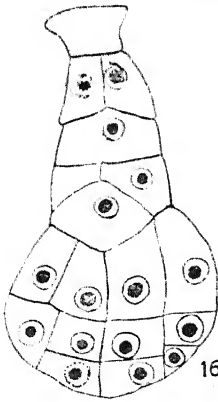*Abnormalities.*—Although the ovule is normally campylotropous three cases were found with completely orthotropous ones (Fig. 18). In one orthotropous ovule, the contents of its 8-nucleate embryo-sac were in a state of complete degeneration. Another ovule in which the endosperm was formed, did not show any embryo. In the third one, also a mature ovule, even the embryo-sac contents could not be found. Perhaps they degenerated early. Nevertheless the ovule continued to grow.

Another interesting abnormality (Fig. 19) is a case where two completely separate nucelli are enclosed by the same integument. Still another interesting case is provided by two separate but closely appressed ovules, each with its own integuments but with a single common funiculus (Fig. 20). The embryo-sacs in these are in a state of degeneration.

Abnormalities are also found in the composition of the ovary. Normally, it is 8-carpellate, but in some cases, the number of carpels varies between 6 and 9.

Capparis sepiaria

Here, the ovary is bicarpellate. The development of the ovule is as in *C. galeata.* Degenerations are extensive. The ovule shown in Fig. 21 is similar to that of *C. galeata* shown in Fig. 5, and contains a mature, but degenerating embryo-sac. Fruits are not available, and therefore the development from this stage onward to the mature form of ovule could not be traced.

The development of the embryo-sac also is mostly similar to to that of *C. galeata.* The parietal tissue is less extensive. In contrast to *C. galeata,* here it is the inner integument that grows much faster than the outer integument (Fig. 22) which markedly lags behind in growth, so that the micropyle is formed only by the inner integument. All the embryo-sacs are full of starch grains. The nucellar tip in many ovules is exposed (Fig. 23) and is not covered properly by the integuments. Degenerations are numerous in the mature embryo-sac.

The egg cell (Fig. 24) lies either at the same level as the synergids or a little higher up. The nucleus lies usually at the centre of the cell and the cytoplasm is faint. Sometimes the cyto-

plasm surrounding the egg nucleus contains numerous small vacuoles. The nucleus of the synergids lies in the basal widened portion but the position is not very constant. The cytoplasm in this region is usually much less dense than elsewhere. A few synergids are observed with a slight development of a beak. In many old synergids, a faint filiform apparatus is found. A filiform apparatus is recognisable only in synergids which are very old and nearing degeneration, the younger ones always lacking it.
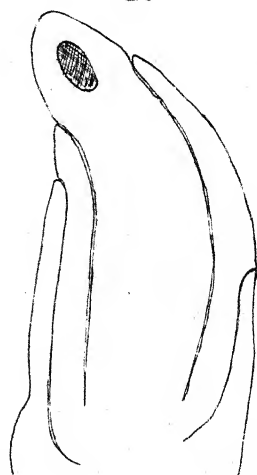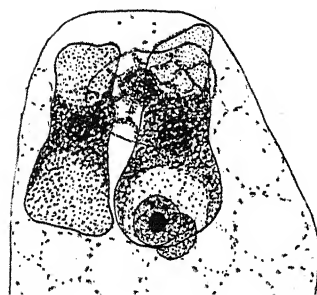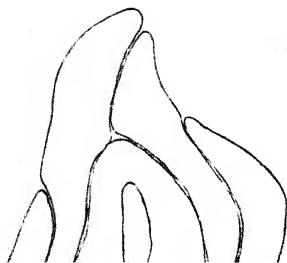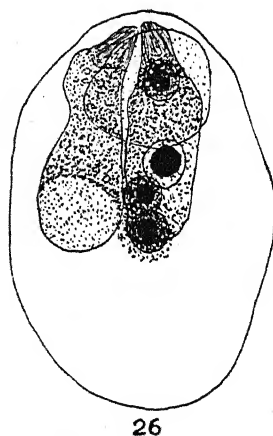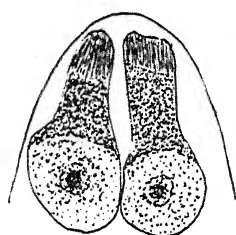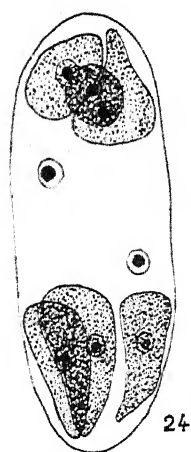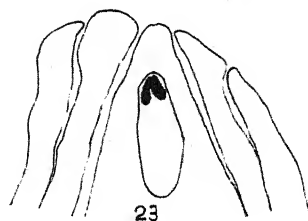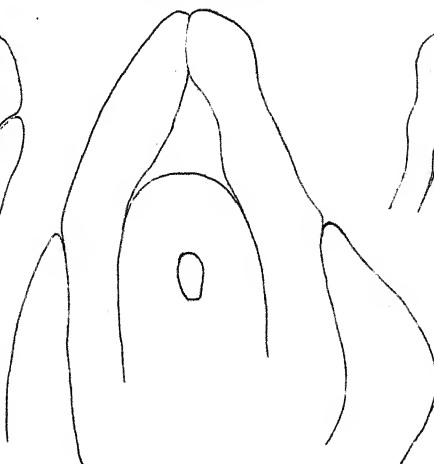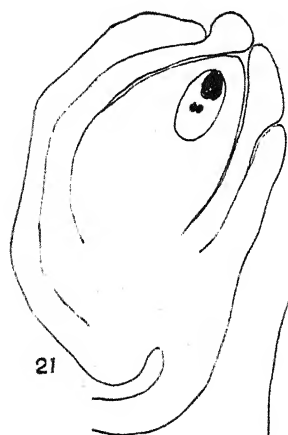
The antipodals (Fig. 24) are somewhat large and wedge-shaped as in *C. galeata* and degenerate very early. The two polar nuclei lie close to each other, and near the egg apparatus (Fig. 26). In every sac, degenerations are universal at this stage, and it has been impossible to get a stage later than this. The degeneration of the two polar nuclei begins earlier than that of the egg cell. By the time the egg shows symptoms of degeneration, the synergids, the antipodals, and the polar nuclei are degenerated. The mature embryo-sac lies about 3 or 4 cells deep within the nucellus. The length of the micropyle varies from nil (in cases where the nucellar tip is completely exposed and uncovered by the integuments) to a long tube-like structure (Fig. 27).

Abnormalities.—An interesting abnormal ovule (Fig. 29) was found in which both the integuments ended far below the tip of the nucellus, the outer integument lagging far behind the inner. A mature embryo-sac, in a state of complete degeneration was found in the completely exposed region of the nucellus. It is rather a common feature in this plant that nucellar tip is slightly exposed, but in this particular ovule, we find this condition very much accentuated. Whether this incomplete growth of the integuments has got anything to do with the very wide degenerations in the embryo-sacs can merely be speculated.

Capparis horrida

Here also, the development of the ovule is essentially similar to that of *C. sepiaria*. But the growth of the integuments is similar to that in *C. galeata* in as much as the outer integument usually grows faster than the inner. Cases are also found where the outer integument slightly lagged behind in growth.

The ovules arise on four parietal placentae. The ovary is unilocular in the young condition and tetralocular when old, due to the ingrowth of tissue from the placental region. This results in a condition similar to that in *C. galeata, i.e.,* some of the ovules come to have their place of origin on the septa (Fig. 30), whose tissue has grown in from the placental region, from among the bases of the funicles. The growth of the integuments is very rapid. The union of the septa in the centre is much closer and more complete than in *C. galeata.*

Fig. 30. T. S. of ...... of C. The ....

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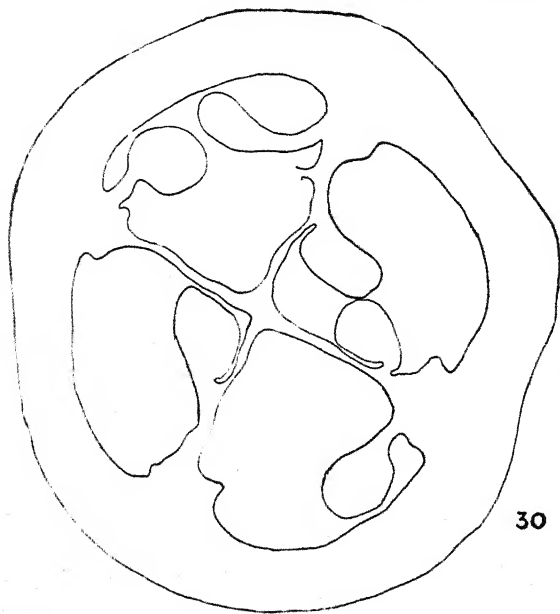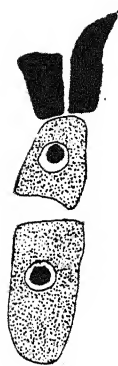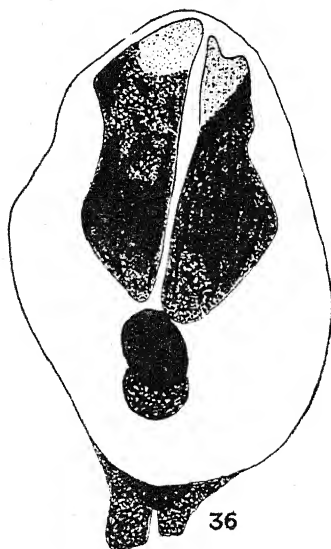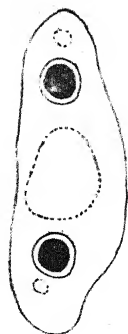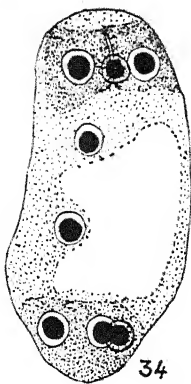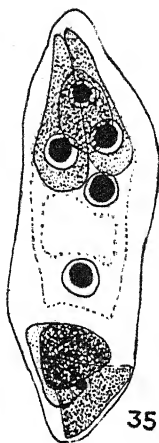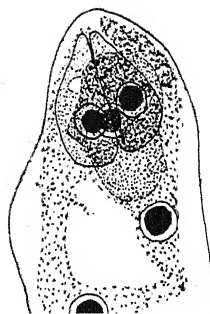
In megasporogenesis, both linear and T-shaped tetrads are found in equally large numbers (Figs. 31 and 32) and it is impossible to say which is of more common occurrence. From the 2-nucleate stage onwards, a large central vacuole is always found (Fig. 33). Moreover, in the 2-nucleate stage, a small vacuole is sometimes found near each nucleus on the outside. The development of the embryo-sac is normal, and the size and structure of the embryo-sac are similar to the condition in *C. sepiaria*.

The synergids (Fig. 35) throughout the greater portion of their life are wedge-shaped, with the nucleus lying towards the base. The completely mature synergids are slightly flask-shaped, and only in the very old stages is found the presence of a faintly developed filiform apparatus (Fig. 36). The life history of all other nuclei of the embryo-sac is identical with that of *C. sepiaria*.

Degenerations are very extensive, and the embryo-sacs are full of starch grains. The starch is markedly developed in the embryo-sacs at the beginning of degenerations. The usual order of degeneration in the embryo-sac is,—antipodals, egg, synergids, and the polar nuclei. This conforms exactly to the order mentioned by D'Hubert (1896) in Cactaceae, as cited by Schnarf (8) (p. 172).

The few points mentioned by Hedayatullah in his abstract upon the embryo-sac development in this plant agree with mine. This abstract, however, makes no mention of many important points, for instance, the appearance of a filiform apparatus in the old stages.

Abnormalities.—Two pentacarpellary and one tricarpellary ovaries were also found. In one embryo-sac, the antipodals are seen to have become slightly elongated (Fig. 38) and protruding into the nucellus on the chalazal side, such as those described by Tiwary (9) in *Cleome viscosa*.

Discussion

All the species of *Capparis* here described show a remarkable uniformity of development and structure in so far as these have been studied. Degenerations are widespread in the 8-nucleate embryo-sac in all the species, starch abounding in the degenerating cells. The appearance of a filiform apparatus in the old stages of synergids in all the species, is also a noteworthy feature. The presence of starch has also been described for *C. rupestris* by Mauritzon (4). For *C. frondosa*, he has also described the occurrence of nucellar embryos. He mentions generally that the outer integument develops more rapidly than the inner, but I find that *C. sepiaria* forms an exception to this. Moreover, he has not described the formation of a filiform apparatus in those two species studied by him.

The structure of the mature embryo-sac of *Capparis* differs slightly from that of *Gynandropsis* and *Cleome*, and approaches

more closely that of *Maerua*. In *Gynandropsis* and *Cleome* the mature compylotropous form of the ovule is reached through the intermediate anatropous form by the curvature at the base of the ovule, but in *Capparis* (refer *C. galeata*) the campylotropous form is reached without this intervention, by a peculiar unilateral growth at the base of the ovule around the point of attachment of the funiculus. In short, in *Gynandropsis* and *Cleome*, bending of the ovule occurs prior to unilateral growth, whereas in *Capparis,* unilateral growth precedes curvature.

The outline of development of the embryo-sac conforms to that described for *Gynandropsis*. The synergids of *Gynandropsis* and *Cleome* are devoid of a filiform apparatus, whereas in those of *Maerua* and *Capparis* it is present. In *Maerua*, it is even much more prominently developed than in *Capparis*. In *Cleome* the antipodals are very small, but in *Maerua* and *Capparis*, they are much larger, occupying a prominent space in the embryo-sac. Although the absolute size of the antipodals in *Capparis* does not equal those of *Maerua*, yet, considering the fact that the size of the embryo-sac of *Maerua* is much larger than that of *Capparis*, it can be affirmed that the antipodals resemble those of *Maerua*, and certainly not those of *Cleome*.

The endosperm is nuclear, and is formed before the first division in the egg cell. In *Gynandropsis* and *Cleome*, the pro-embryo consists of only 3 or 4 cells, whereas in *C. galeata*, it consists of 7 or 8 cells. In the latter plant, longitudinal divisions in some of the suspensor cells are also observed, whereas such a condition is not found in *Gynandropsis*.

The differences in characters between *Maerua* and *Capparis* on the one hand, and *Gynandropsis* and *Cleome* on the other, definitely support the present arrangement, [Hooker (3)] of *Cleome* and *Gynandropsis* under the tribe *Cleomae*, and *Capparis* and *Maerua*, along with others under Capparae, as suggested by Mauritzon (4).

Summary

C. galeata

(1) The development of the embryo-sac is normal, and in mature synergids, a filiform apparatus is found.

(2) In the development of the embryo, some of the suspensor cells also divide and contribute to the formation of the embryonal sphere of cells.

(3) The mature ovule is Campylotropous, but the orthotropous condition is found as an abnormality. Other interesting abnormalities are also found.

C. sepiaria

(1) The development of the embryo-sac is similar in essentials to that of *C. galeata*.

(2) In an abnormality, the integuments end far below the tip of the nucellus.

C. horrida

The development is similar to *C. sepiaria*. Degenerations in the embryo-sacs abound in all the species, and starch is found in the degenerating cells.

I wish to express my heart-felt thanks to Prof. N. K. Tiwary for his kind and most helpful guidance during the investigation and also for going through the manuscript and making valuable suggestions. I also thank Dr. Y. Bharadwaja of Benares Hindu University and Dr. S. C. Nandimath of Lingaraj College, Belgaum, for giving facilities for research and preparation of the MS.

Literature Cited

1. GUIGNARD, L. Recherches sur le developpement de la graine et an particulier du tegument seminal; Joura de Bot, 7, 1883 (cited by Schnarf).

2. HEDAYATULLAH. The embryo-sac development in *Capparis horrida*, L. F.—Abstracts Ind. Sc., Cong., Calcutta, 1935.

3. HOOKER, J. D. The flora of British India, Vol. I.

4. MAURITZON, J. Die Embryologie einiger capparidaceae sowie von Tovaria pendula. Arkiv fuer Bot. 26, 1934.

5. RAO, V. S. Studies on capparidaceae I. The embryo-sac of *Maerua arenaria*. Journ. Ind. Bot. Soc. 15, 1936.

6. RAO, V. S. Studies on Capparidaceae II. The embryology of *Gynandropsis pentaphylla*. Journ. Ind. Bot. Soc. 15, 1936.

7. SCHILLER, J. Uber den verlauf der kernteilung bei *capparis* mit dauer chromosomen Jahrb. F. Wiss. Bot. 69, 1928.

8. SCHNARF, K. Embryologie der Angiospermen.

9. TIWARY, N. K. Megasporogenesis and embryo development in *Cleome viscosa*, Linn. Science and Culture I, 1936.

THE MYXOPHYCEAE OF THE MADRAS PRESIDENCY, INDIA—I*

BY

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During the years 1934-1937, the writer made several collections of algae from the Madras Presidency, mainly from Vizagapatam, Cocanada, Ellore, Dendulur, Bhimavaram, Polamur, Namdur, Veeravasaram and Palacol and also from Chittavaram, Narsapur and Mogaltur, together with their environs. It is now desired to record a systematic account of these algae in a series of papers. This communication deals with some of the Myxophyceae. In all seventy forms representing twenty-two genera, have been described and out of these two species and seven forms are new.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED

I. Chroococcales

CHROOCOCCACEAE

Genus *Microcystis* Kuetzing

1. *Microcystis flos-aquae* (Wittr.) Kirchn. Smith, Ecology of the Plankton Algae of the Palisades Interstate Park, *Roosevelt Wild Life Bulletin*, Vol. 2, No. 2, 1924, Pl. 3, Fig. 13; Geitler, in *Rabenhorst's Kryptogamenflora von Europa*, Band XIV, Cyanophyceae, 1930-32, p. 136, Fig. 59 *e* and *f*.

Lat. cell., 3–6 μ.

Habitat:—Narsapur: in a tank; Polamur and Ellore: in temple tanks; Cocanada: in a road-side pond, (J. Venkateswarlu).

Genus *Aphanocapsa* Naegeli

2.   *Aphanocapsa pulchra* (Kütz.) Rabenh.   Geitler, *op. cit.*, 1930–32 p. 156, Fig. 69 g; Frémy, Les Myxophycées de l'afrique équatoriale française, *Archives de Botanique*, iii. (1929), Mem. 2 1930, p. 23, Fig. 22.

Lat. cell., 3·8–4·8 µ.

Habitat :—Namdur : free-floating in a pond.

Genus *Aphanothece* Naegeli

3   *Aphanothece pallida* (Kütz.) Rabenh.   Geitler, *op. cit.*, 1930–32, p. 172, Fig. 78; Frémy, *op. cit.*, 1930, p. 33, Fig. 31.

Lat. cell., 5–6·9 µ; long. cell., 6·6–10 µ.

Habitat :—Narsapur, Ellore and Cocanada : on moist soil ; Polamur and Namdur : in stagnant water of rice-fields, along with *Oscillatoria calcuttensis* forma.

4.   *Aphanothece bullosa* (Menegh.) Rabenh.   Frémy, *op. cit.*, 1930, p. 33, Fig. 33.

Lat. cell., 4·5–5·2 µ; long. cell. 6–10·4 µ.

Habitat :—Namdur : floating in stagnant ponds, along with *Oscillatoria calcuttensis* forma, *Chara* sp., *Azolla* sp., and *Nelumbium* sp.

5.   *Aphanothece microscopica* Nag. Geitler, *op. cit.*, 1930–32, p. 173, Fig. 79.

Lat. cell., 4–5 µ; long. cell., 6–8 µ.

Habitat :—Ellore : on soil submerged in water at the edge of a pond along with moss and grass seedlings.

Genus *Chroococcus* Naegeli

6.   *Chroococcus türgidus* (Kütz.) Nag. Geitler, *op. cit.*, 1930–32, p. 228, Fig. 109 b; Frémy, *op. cit.*, 1930, p. 41, Fig. 40; Tilden, Minnesota Algae, Vol. I, 1910, Pl. I, Fig. 3; West, Algae, Vol. I, 1916, p. 41, Fig. 25 b.

Lat. cell., 8·2–13·2 µ; long. cell., 6·6–10 µ; lat. colon. cum mem., 13–30 µ; long. colon. cum mem., 16–38 µ.

Habitat :—Namdur : in a road-side pond, along with *Oscillatoria prolifica, Tolypothrix robusta* forma and *Oscillatoria princeps.*

7.   *Chroococcus pallidus* Nag.   Geitler, *op. cit.*, 1930–32, p. 239, Fig. 116 b; Frémy, *op. cit.*, 1930, p. 41, Fig. 48.
     Forma.

Stratum thick, slimy and membranous.   Cells almost spherical or ellipsoidal; single or in more or less rounded or ellipsoidal colonies of 2–8 or sometimes upto 16–24 cells, when they overlap. Sheath thick, hyaline and unstratified.

Lat. cell., 3·2–4·8 (–6) *μ*; long. cell., 4–8 *μ*; lat. cell. cum mem., 4·8–6·6 *μ*; long. cell. cum mem., 9–10 *μ*; lat. colon. cum mem., 8–20 *μ*; long. colon. cum mem., 10–26 *μ*.

Habitat:—Namdur: on the inner side of a brick enclosure of a well, along with moss.

The form differs from the type in having smaller cells, that are sometimes 16–24 in each colony overlapping each other.

Genus *Merismopedia* Meyen

8. *Merismopedia minima* G. Beck. Bhashyakarla Rao, The Myxophyceae of the United Provinces, India—II, *Proceedings of the Indian Academy of Sciences*, Vol. III, No. 2, Sec. B, 1936, p. 166, Fig. 1, *B*.

Lat. cell., 0·5–0·6 *μ*.

Habitat:—Ellore: in a fountain tank of a private garden, along with *Merismopedia tenuissima* and others.

9. *Merismopedia tenuissima* Lemm. Geitler, *op. cit.*, 1930–32, p. 264, Fig. 129 *b*; Frémy, Les Cyanophycées des Côtes d'Europe, *Mémoires de la Société Nationale des Sciences Naturelles et Mathématiques de Cherbourg*, tome XLI, 1934, Pl. I, Fig. 1; Geitler, in Pascher's *Süsswasser-flora Deutschlands, Osterreichs und der Schweiz*, Heft 12, Cyanophyceae, 1925 p. 107, Fig. 123 *a*.

Lat. cell., 1·3–2 *μ*.

Habitat:—Cocanada: in stagnant water of low-lying areas; Ellore: in a fountain tank of a private garden, along with *Merismopedia minima* and others; by the side of the road connecting Polamur and Veeravasaram: in a stagnant pond, along with *Gloeotrichia Raciborskii* var. *conica*.

II. Chaemosiphonales

1. DERMOCARPACEAE

Genus *Stichosiphon* Geitler

10. *Stichosiphon indica* Rao. Bhashyakarla Rao, A new species of *Stichosiphon* (*Stichosiphon indica* sp. nov.), *Proceedings of the Indian Academy of Sciences*, 1935, B, Vol. 2, No. 6, p. 536, Figs. 1–10.

Lat. sporang., 6–8·8 *μ*; long. sporang., 50–230 and rarely upto 300 *μ*; lat. spor. cylindric., 5·5–7·5 *μ*; long. spor. cylindric., 5–14 *μ*; diam. spor. spheric., 4·8–7·8 *μ*; crass. vag., upto 0·8 *μ*.

Habitat:—Ellore and Chittavaram: in rain-water pools epiphytic on species of *Cladophora* growing on snails; Namdur: epiphytic on *Rhizoclonium* sp. growing in a pond; Cocanada: epiphytic on *Cladophora* sp. growing in a pond (J. Venkateswarlu).

2. CHAEMOSIPHONACEAE

Genus *Chaemosiphon* A. Braun and Grunow

11. *Chaemosiphon sideriphilus* Starmarch var. *glabra* Rao. Bhashyakarla Rao, The Myxophyceae of the United Provinces, India—III, *Proceedings of the Indian Academy of Sciences,* 1937, Sec. B, Vol. 6, No. 6, p. 348, Fig. 2, *B*.

Lat. sporang., $2 \cdot 4$–$3 \cdot 4$ μ; long. sporang., $4 \cdot 8$–$14 \cdot 4$ μ; lat. sporang. cum mem., 3–$3 \cdot 5$ μ; long. sporang. cum mem., $12 \cdot 8$–$18 \cdot 4$ μ; lat. exospor., 3–$3 \cdot 2$ μ.

Habitat:—Dendulur: on *Rhizoclonium* sp. growing in a pond along with flowering plants; Polamur: on *Lyngbya gracilis* adhering to the steps submerged in the water of a pond.

III. Hormogoneales

1. RIVULARIACEAE

Genus *Calothrix* Agardh

12. *Calothrix Elenkinii* Kossinskaja. Geitler, *op. cit.,* 1930-32, p. 608, Fig. 383 5 and 6.

Forma.

Lat. fil., at base $9 \cdot 6$–$11 \cdot 2$ μ, at the top $6 \cdot 6$–8 μ; lat. trich., at the base $4 \cdot 8$–$8 \cdot 8$ μ, at the top 3–4 μ; long. cell., $1 \cdot 6$–$4 \cdot 8$ μ; lat. het., $4 \cdot 8$–$7 \cdot 2$ μ; crass. vag., upto $1 \cdot 6$ μ.

Habitat:—Polamur: on a log of wood floating at the edge of a pond.

The form is characterised by the slightly broader trichomes and the thicker sheath.

13. *Calothrix Viguerii* Frémy. Frémy, *op. cit.,* 1930 p. 252, Fig. 226.

Forma.

Lat. fil., at the base 10–22 μ, at the top $6 \cdot 6$–10μ; lat. trich., at the base 10–16 μ, at the top $2 \cdot 5$–$5 \cdot 5$ μ; long. cell., at the base 3–10 μ, at the top 28–50 μ; lat. het., 8–16 μ; long. het., 5–11 μ.

Habitat:—Polamur: in a road-side pond, along with *Gloeotrichia Raciborskii* var. *conica.*

The form agrees with the type in all respects except that the cells are sometimes longer with granules near the septa and the heterocysts are enclosed by the sheath.

14. *Calothrix brevissima* G. S. West. West, Report on the Fresh-water Algae including Phytoplankton, of the Third Tanga-

nyika Expedition conducted by Dr. W. A. Cunnington. 1904–1905, *Journal of the Linnean Society*, Bot., 1907, 38, p. 180, Pl. 10, Fig. 8.

Lat. fil., 4·8–5·2 μ; long. fil., 20–80 μ; lat. trich., 3·8–4·2 μ; long. trich., 17–60 μ; long. cell., 1·6–3·5 μ; lat. het., 4–4·8 μ; long. het., 4·2–5·6 μ.

Habitat:—Namdur: Epiphytic on *Lyngbya gracilis* adhering to a wooden log at the edge of a pond; epiphytic on *Lyngbya majuscula* var. *chakiaense* growing in a pond.

Genus *Gloeotrichia* Agardh

15. *Gloeotrichia Raciborskii* Woloszynska var. *conica* Dixit. Dixit, The Myxophyceae of the Bombay Presidency. India—I, *Proceedings of the Indian Academy of Sciences*, 1936, B. Vol. III, No. 1, p. 96, Fig. 1, *F* and *G*.

Diam. fil., 26–40 μ; lat. trich., at the base 6·6–9 μ, at the top upto 3·3 μ; long. cell., at the base 3·3–4·8 μ; at the top 11–26 μ; lat. het., 10–13 μ; lat. spor., 10–16 μ; long. spor., 40–70 μ.

Habitat:—Namdur: in a road-side pond, along with *Calothrix Viguerii* forma. This alga has sometimes bigger spores than those of the Bombay form.

16. *Gloeotrichia natans* Rabenh. Geitler, *op. cit.*, 1930–32, p. 638, Fig. 406; Frémy, *op. cit.*, 1930, p. 277, Fig. 246.

Lat. trich., at the base 6·6–8·2 μ; higher up 5–7·5 μ; long. cell., at the base 4–8 μ, higher up 5–22 μ; lat. het., 10–11 μ; lat. spor., 10–13 μ; long. spor., 40–52 μ; lat. spor. cum vag., 23–33 μ.

Habitat:—Namdur and Polamur: in water-logged rice-fields.

Genus *Rivularia* Agardh

17. *Rivularia globiceps* G. S. West. West, *op. cit.*, 1907, p. 182, Fig. 6.

Forma.

Lat. trich., at the base, 3·2–4 μ higher up 0·8–1 μ; long. cell., at the base 3·2–11·2 μ, higher up 2·4–3·2 μ; lat. het., 4·8–8 μ; long. het., 6·4–9·6 μ.

Habitat:—Vizagapatam: in a stagnant puddle on a hillock extending into the sea.

But for the narrower trichomes and smaller heterocysts, the form agrees with the type in all respects.

2. MICROCHAETACEAE

Genus *Aulosira* Kirchin.

18. *Aulosira Fritschii* Bhâradwâja. Bhâradwâja, Contributions to our knowledge of the Myxophyceae of India, *Annals of Botany*, XLVII, 1933, pp. 123–131, Figs. 3 and 4.

2

Lat. fil., 15·8–16·5 μ; crass. vag., 1·6–2·2 μ; lat. trich., 10–12·3 μ; lat. het., 12·8–13·2 μ; long. het., 11·2–19·8 μ; lat. spor., 11·2–12·8 μ; long. spor., 9·9–33 μ.

Habitat:—Ellore: in a pond along with *Oedogonium* sp.; Chittavaram: in a pond, along with *Aulosira fertilissima* var. *tenuis, Oscillatoria Hamelii, Lyngbya confervoides* and others.

19. *Aulosira prolifica* Bhâradwâja. Bhâradwâja, *op. cit.,* 1933, pp. 131–136, Figs. 5 and 6.

Lat. fil., 4·8–5·6 μ; lat. trich. 3·2–4·8 μ; long. cell., 8–16 μ; lat. het., 4–6·4 (–8) μ; long. het., 4·8–16 μ.

Habitat:—Ellore: in a pond, along with aquatic angiosperms.

20. *Aulosira fertilissima* Ghose var. *tenuis* Rao. Bhashyakarla Rao, *op. cit.,* 1937, p. 352, Fig. 3, *F* to *I*.

Lat. fil., 4·8–10 μ; crass. vag., upto 3 μ; lat. trich., 3·3–5·5 μ; long. cell., 4·8–10 μ; lat. het., 4·8–6 μ; long. het., 8·2–16·5 μ; lat. spor., 6·6–10 μ; long. spor., 13·2–19·8 μ.

Habitat:—Chittavaram: in a pond, along with *Aulosira Fritschii, Oscillatoria Hamelii* and *Lyngbya confervoides;* Namdur: in a road-side pond, along with *Anabaena unispora* var. *crassa.*

3. Scytonemataceae

Genus *Plectonema* Thuret

21. *Plectonema indica* Dixit. Dixit, *op. cit.,* 1936, p. 98, Fig. 2, *E* and *F*.

Forma.

Lat. fil., 8·2–13 μ; lat. trich., 6·4–8 μ; long. cell., 3·3–16 μ; crass. vag., upto 1·5 μ.

Habitat:—Narsapur: on a damp chunam wall in the writer's house. The sheath in this form is always thin and unstratified.

Genus *Tolypothrix* Kuetzing

22. *Tolypothrix nodosa* Bhâradwâja. Bhâradwâja, The Taxonomy of *Scytonema* and *Tolypothrix* including some new records and new species from India and Ceylon. *Revue Algalogique,* 1933, n. 1–2, p. 176, Fig. 7 *c*.

Lat. fil., 5·5–7·2 μ; lat. trich., 4–5·2 μ; long. cell., 4–20 μ; lat. het., 4·8–8·2 μ; long. het., 10–16·5 μ.

Habitat:—Namdur: in a pond, along with *Anabaena unispora* var. *tenuis* and other aquatic angiosperms; Polamur: in stagnant ponds.

23. *Tolypothrix robusta* Gardner forma Rao. Bhashyakarla Rao, *op. cit.,* 1937, p. 354.

Lat. fil., 13·2–14·8, when old upto 20 μ; crass. vag., 2–5 μ, when old upto 6 μ; lat. trich., 8–13, when old and unhealthy

narrowed down to 6·6 μ; long. cell., 8–12 μ, when old and unhealthy upto 40 μ; lat. het., 6·6–10 μ; long. het., 11–36 μ.

Habitat:—Polamur: in a pond; Namdur: in a road-side pond. along with *Lyngbya confervoides* and *Oscillatoria princeps;* in a rain-water puddle by the side of the rail-way line connecting Palacol and Narsapur.

Genus *Scytonema* Agardh

24. *Scytonema Fritschii* Ghose. Ghose, A systematic and an ecological account of Blue-green Algae of Lahore and Simla. *Journal of the Linnean Society*, Bot., 1923, XLVI, p. 342, Pl. 31, Fig. 11; Bhâradwâja, The Taxonomy of *Scytonema* and *Toly-pothrix* including some new records and new species from India and Ceylon, *Revue Algologique*, 1933, n. 1–2, p. 157.

Lat. fil., 16·5–19 (–22) μ; crass. vag., 3·3–4·8 μ; lat. trich., 6·6–13·2 μ; long. cell., 3·3–23 μ; lat. het., 13–15 μ; long. het., 23–46 μ.

Habitat:—Ellore: in a pond.

The present alga agrees with the second of the two forms collected by M. O. P. Iyengar from the Vandalur Tank, Madras, and described by Bhâradwâja.

25. *Scytonema coactile* Mont. Geitler, *op. cit.*, 1930–32, p. 753, Fig. 479.

Lat. fil., 16·5–19·8 μ; crass. vag., 3·3–4 μ; lat. trich., 10–11·8 μ; long. cell., 10–16·5 μ; lat. het., 13·2–16·5 μ; long. het., 16·5–23 μ.

Habitat:—Palacol: on stones submerged in water at the edge of a pond.

26. *Scytonema guayanense* (Mont.) Born. et Flah. Frémy, *op. cit.*, 1930, p. 312, Fig. 265.

Lat. fil., (13·2–) 15–16·5, when old upto 20 μ; crass. vag., 2–3, when old upto 4 and thinned out at apices to 1 μ; lat. trich., 11–14, when old and unhealthy narrowed down to 9 μ; long. cell., 7–20 μ; lat. het., 10–14 μ; long. het., 11–20 μ.

Habitat:—Polamur: on damp brick work near a well.

27. *Scytonema mirabile* (Dillw.) Born. Geitler, *op. cit.*, 1930–32, pp. 776 and 777, Figs. 498 a–f; Frémy, *op. cit.*, 1930, p. 318, Fig. 268; Bhâradwâja, The Taxonomy of *Scytonema* and *Tolypothrix* including some new records and new species from Ceylon, *Revue Algologique*, 1933, n. 1–2, p. 171, Fig. 5 *A*.

Lat. fil., 13–16 μ; crass. vag., 3–4·5 μ; lat. trich., 5–7·2 μ; long. cell., 3–8 μ; lat. het., 6·6–8·2 μ; long. het., 10–13·2 μ.

Habitat:—Namdur: on the bricks of a well pavement.

4. Nostocaceae

Genus *Cylindrospermum* Kuetzing

28. *Cylindrospermum muscicola* Kütz. Frémy, *op. cit.,* 1929, p. 377, Fig. 313; Tilden, *op. cit.,* 1910, Pl. X, Fig. 6.

Lat. cell., 2·8–3·3 μ; long. cell., 2·8–4·8 μ; lat. het., 4–4·2 μ; long. het., 4·8–6·6 μ; lat. spor., 8·5–11·5 μ; long. spor., 13·2–19·8 μ.

Habitat:—Polamur: in a pond intermingled with *Chara* sp.

Genus *Nostoc* Vaucher

29. *Nostoc carneum* Ag. forma *minor* Bhâradwâja. Bhâradwâja, The Myxophyceae of the United Provinces, India—I, *Proceedings of the Indian Academy of Sciences,* 1935, B, Vol. II, No. 1, p. 102; Geitler, *op. cit.,* 1930–32, p. 839, Fig. 530.

Lat. cell., 3·2 μ; long. cell., 3·2–6·4 μ; lat. het., 4–6·4 μ; long. het., 4–7·2 μ; lat. spor., 4·8–6·4 μ; long spor., 6·4–9·6 μ.

Habitat:—Bhimavaram: on wet soil near a water course amidst crop-fields.

30. *Nostoc spongiaeforme* Ag. var. *tenuis* Rao. Bhashyakarla Rao, *op. cit.,* 1936, p. 168, Fig. 2 *F*.

Lat. cell., 3·2–3·8 μ; long. cell., 3·2–5·6 μ; lat. het., 4·8–5·6 μ; long. het., 4·8–6·4 μ; lat. spor., 4·8–5·5 μ; long. spor., 4·5–8 μ.

Habitat:—Ellore: on moist soil.

31. *Nostoc ellipsosporum* Rabenh. Geitler, *op. cit.,* 1930–32, p. 842, Fig. 533.

Lat. cell., 3·8–4·2μ; long. cell., 5·6–8·8 μ; lat. het., 4–6·4 μ; long. het., 6·4–9·6 μ; lat. spor., 6·4–8 μ; long. spor., 8–12 μ.

Habitat:—Mogaltur: in salt-water puddles, along with *Oscillatoria nigroviridis* and *Spirulina major*.

The spores in this form are somewhat shorter than those of the type.

Genus *Anabaena* Bory

32. *Anabaena unispora* Gardner var. *crassa* Rao. Bhashyakarla Rao, *op. cit.,* 1937, p. 360, Fig. 5, *D* and *E*.

Lat. cell., 4·8–5·8 μ; long. cell., 4·8–16·5 μ; lat. het., 5–6·6 μ; long. het., 6·6–14·8 μ; lat. spor., 10–13·2 μ; long. spor., 26–35 μ.

Habitat:—Namdur: in a pond, along with *Tolypothrix nodosa*.

5. OSCILLATORIACEAE

Genus *Spirulina* Turpin

33. *Spirulina subsalsa* Oerst.   Geitler, *op. cit.*, 1930–32, p. 928, Fig. 593 *a;* Tilden, *op. cit.*, 1910, Pl. IV, Fig. 49.

Lat. trich., 1·5–2 *μ*; lat. spor., 3·8–5 *μ*.

Habitat:—Palacol: on water logged soil at the edge of a tank (Ram Gundam) along with *Oscillatoria formosa;* Polamur: floating in ponds, along with *Spirulina major, Oscillatoria formosa* and *O. princeps.*

34. *Spirulina major* Kütz.   Geitler, *op. cit.*, 1930–32, p. 930, Fig. 595; Frémy, *op. cit.*, 1930, p. 235, Fig. 208; Tilden, *op. cit.*, 1910, Pl. IV, Fig. 46; Frémy, *op. cit.*, 1934, Pl. 31, Fig. 18; Carter, A comparative study of the algal flora of two salt marshes, Part II, *Journal of Ecology*, Vol. XXI, i, 1933, p. 159, Fig. 2; Ghose, On some Myxophyceae from Rangoon, *Journal of the Burma Research Society*, Vol. XV, Part III, 1926, Pl. VI, Fig. 3.

Lat. cell., 1·2–1·5 *μ*; lat. spir., 2·5–3·5 *μ*; spat. inter duo spir., 2·4–6 *μ*.

Habitat:—Ellore: in a pond along with *Oscillatoria quadri-punctulata* and others; Narsapur: in a pond along with *Oscillatoria chalybea, O. terebriformis* forma and *O. princeps;* Mogaltur: in salt water puddles near salt creaks, along with *Oscillatoria nigro-viridis,* and *Nostoc ellipsosporum;* Polamur: floating in ponds, along with *Spirulina subsalsa, Oscillatoria princeps* and *O. formosa.*

Genus *Oscillatoria* Vauch.

35. *Oscillatoria vizagapatensis* sp. nov. (Figs. 1–3).

Plant-mass blue-green.   Trichomes straight or bent, pale blue-green, uniformly broad except at the extreme apex, without constrictions at the joints.   Cells much shorter than broad with granular contents; end-cell broadly rounded forming a cap with a slightly thickened outer-wall.

Lat. trich., 8–10 *μ*; long. cell., 1·6–2 *μ*.

Habitat:—Vizagapatam: on moist rocks in a dark and shady cave in a hillock.

The alga agrees with *Oscillatoria limosa* Ag. and *O. obtusa* Gardner in having non-attenuating trichomes without constrictions at the septa but differs from both these species in the presence of a definite cap with a slightly thickened outer wall at the apex of the trichomes, that are much narrower and possess much shorter cells.   It further differs from *O. limosa* in the absence of granules on either side of the septa.

36. *Oscillatoria nigro-viridis* Thwaites. Tilden, *op. cit.*, 1910, Pl. IV, Fig. 12.

Lat. cell., 7·2–9·6 μ; long. cell., 2·4–4 μ.

Habitat:—Mogaltur: in salt-water puddles near salt creaks, along with *Spirulina major* and *Nostoc ellipsosporum*.

37. *Oscillatoria ornata* Kütz. Geitler, *op. cit.*, 1930–32, p. 945, Fig. 599 a.

Lat. cell., 9·8–11·5 μ; long. cell., 3·3–6 μ.

Habitat:—Polamur: in a pond, along with *Oscillatoria formosa, Spirulina subsalsa* and *S. major*.
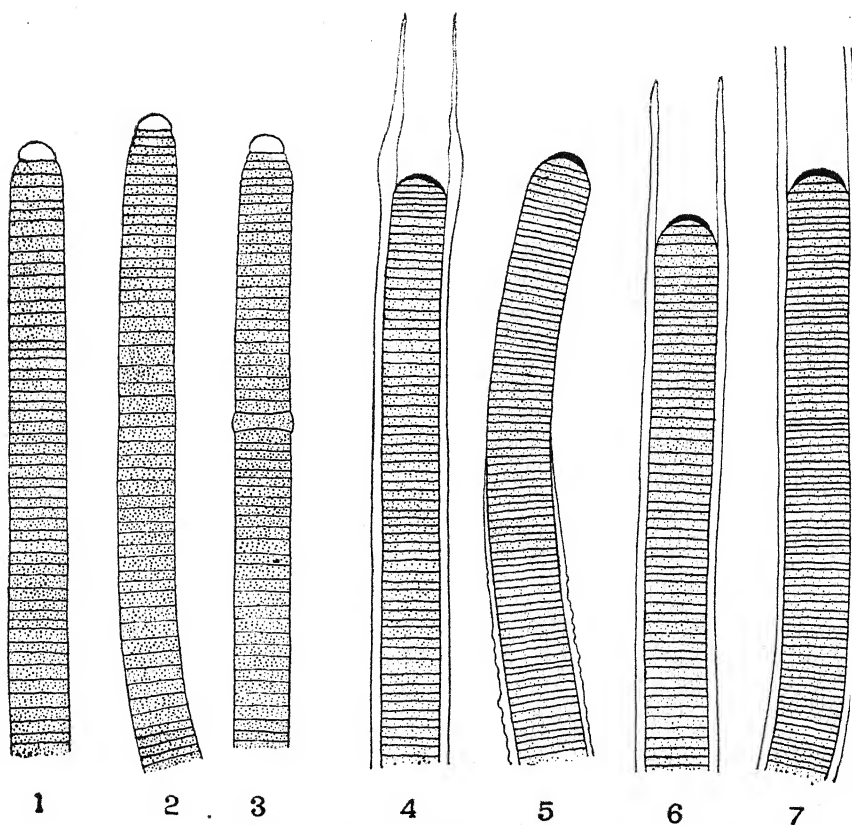
Figs. 1-3.—*Oscillatoria vizagapatensis* sp. nov. Figs. 4-7.—*Phormidium stagnina* sp. nov. All × 875.

38. *Oscillatoria princeps* Vauch. Frémy, *op. cit.*, 1930, p. 207, Fig. 175; Tilden, *op. cit.*, 1910, Pl. IV, Fig. 3.

Lat. trich., 19·8–26 μ; long. cell., 3·5 μ.

Habitat:—Narsapur: in a pond, along with *Oedogonium* sp.; Polamur: free-floating in ponds; Ellore: free-floating in a rain-water puddle amidst rice-fields; Palacol: in waste water drains, along with *Oscillatoria formosa;* Namdur: in a road-side pond, along with *Tolypothrix robusta* forma and *Lyngbya confervoides;* Chittavaram: in a stagnant pond.

The form collected in Chittavaram shows the following measurements: lat. cell., 40–42 μ and long. cell., 4·8–6·6 μ.

39. *Oscillatoria anguina* (Bory.) Gom. Geitler, *op. cit.,* 1930–32, p. 945, Fig. 599 *b.*

Lat. cell., 6·6–8·2 μ; long. cell., 1·5–2·5 μ.

Habitat:—Polamur: in a pond, along with *Phormidium Retzii, Oscillatoria claricentrosa* forma, *Chara* sp. and *Elodea* sp.

40. *Oscillatoria terebriformis* Ag. forma Rao. Bhashyakarla Rao, *op. cit.,* 1936, p. 171; Tilden, *op. cit.,* 1910, Pl. IV, Fig. 39.

Lat. cell., 4·8–6 μ; long. cell., 3·3–5·5 μ.

Habitat:—Narsapur: on soil in the first instance but later free-floating in a pond, along with *Oscillatoria chalybea* and *O. princeps.*

41. *Oscillatoria chalybea* Mertens. Tilden, *op. cit.,* 1910, Pl. IV, Fig. 36.

Lat. cell., 8–10·8 μ; long. cell., 3·3–6·6 μ.

Habitat: Narsapur: on soil in the first instance but later free-floating in a pond along with *Oscillatoria terebriformis* forma and *O. princeps.*

42. *Oscillatoria Hameli* Frémy. Frémy, *op. cit.,* 1930, p. 221, Fig. 187.

Lat. trich., 4·8–5 μ; long. cell., 5–9·6 μ.

Habitat:—Chittavaram: in a pond, along with *Aulosira fertilissima* var. *tenuis, A. Fritschii,* and *Lyngbya confervoides.*

43. *Oscillatoria quadripunctulata* Brühl et Biswas. Brühl et Biswas, The algae of the Bengal Filter Beds, *Journal of the Department of Science,* Calcutta University, 4, 1922, p. 5, Pl. I, Fig. 6; Biswas, Road slimes of Calcutta, *Journal of the Department of Science,* Calcutta University, 7, 1925, p. 10, Pl. II, Figs. 11 *a–d.*

Lat. cell., 1·6–1·8 μ; long. cell., 4–8 μ.

Habitat:—Ellore: in a pond, along with *Spirulina major* and others.

In this form there is one granule on either side of the septum.

44. *Oscillatoria formosa* Bory. Geitler, *op. cit.,* 1930–32, p. 971, Fig. 619 *b.*

Lat. cell., 4·5–6·6 μ; long. cell., 3·3–5 μ.

Habitat:—Palacol: on water-logged soil and in waste-water drains along with *Oscillatoria princeps;* Polamur: in ponds, along with *Spirulina subsalsa* and *S. major.*

Forma.

Lat. cell., 3·8–4·8 *μ*; long. cell., 1–2·4 *μ.*

Habitat:—Narsapur: on moist soil, along with *Oscillatoria animalis* forma *tenuior.*

This form is characterised by having shorter cells.

45. *Oscillatoria claricentrosa* Gardner forma *bigranulata* Rao. Bhashyakarla Rao, *op. cit.,* 1937, p. 370, Fig. 7 *c.*

Lat. cell., 2–2·4 *μ*; long. cell., 4·8–9·6 *μ.*

Habitat:—Chittavaram: in a rain-water puddle along with *Oscillatoria princeps.*

46. *Oscillatoria rubescens* D. C. forma Rao. Bhashyakarla Rao, *op. cit.,* 1937, p. 367.

Lat. cell., 4·8–6·4 *μ*; long. cell., 2·4–4 *μ.*

Habitat:—Polamur: on stones at the edge of a pond.

47. *Oscillatoria prolifica* (Greville) Gomont. Tilden, *op. cit.,* 1910, Pl. IV, Fig. 1.

Lat. cell., 4–5 *μ*; long. cell., 2·4–6 *μ.*

Habitat:—Namdur: in a road-side pond, along with *Oscillatoria princeps, Tolypothrix robusta* forma and *Chroococcus turgidus.*

48. *Oscillatoria calcuttensis* Biswas. Biswas, *op. cit.,* 1925, Pl. I, Fig. 9.

Forma.

Lat. cell., 1·9–2·4 *μ*; long. cell., 2·4–8 *μ.*

Habitat:—Namdur, Polamur and Palacol: in the stagnant water of rice-fields along with *Aphanothece pallida;* Palacol: floating in stagnant ponds, along with *Aphanothece bullosa.*

The trichomes of this form are single, bent or tortuous, with usually straight ends and the granules on either side of the septum varying from one to four.

49. *Oscillatoria animalis* Ag. forma *tenuior* Stockmayer. Geitler, *op. cit.,* 1930–32, p. 950, Fig. 603 *e.*

Lat. cell., 1·8–2·5 *μ*; long. cell., 2–4·8 *μ.*

Habitat:—Narsapur: on moist soil along with *Oscillatoria formosa* forma.

Genus *Phormidium* Kütz.

50. *Phormidium stagnina* sp. nov. (Figs. 4–7).

Thallus soft, blue-green and membranous. Trichomes blue-green, laxly interwoven and non-attenuating. Sheath hyaline, unstained with Chlor-zinc-iodide, thick, firm, sometimes diffluent or dissolved. Cells small, without constrictions at the joints. End-cell broadly rounded with a prominent calyptra.

Lat. fil., 12·8–14·4 μ; lat. cell., 8–9·6 μ; long. cell., 1·3–2 μ.

Habitat:—Palacol: in stagnant ponds along with aquatic angiosperms.

The alga can be compared to *Phormidium olivascens* Frémy and *P. pachydermaticum* Frémy in the presence of non-attenuating trichomes without constrictions at joints and provided with a sheath, that does not turn blue with Chlor-zinc-iodide. But it differs from both these species in the broader trichomes, shorter cells and in the end-cells being broadly rounded and provided with a prominent calyptra. It further differs from the former species in the presence of a blue-green soft membranous stratum with laxly interwoven filaments and from the latter in the sheath being unstratified.

51. *Phormidium Bohneri* Schmidle. Schmidle, "Beitrage Zur Algenflora Afrikas", *Engler's Botanische Jahrbucher*, 30, 1902, Pl. II, Fig. 11.

Lat. cell., 1·7–2 μ; long. cell., 1–2·4 μ.

Habitat:—Palacol: on stones near a house outlet, along with *Aphanocapsa montana;* Narsapur: on steps submerged in the water of a tank.

52. *Phormidium cebennense* Gom. Frémy, *op. cit.*, 1930, p. 147, Fig. 129.

Lat. cell., 1·8–2 μ; long. cell., 1–1·6 μ.

Habitat:—Polamur: on steps submerged in water of a pond, along with *Lyngbya gracilis*.

53. *Phormidium valderianum* (Delp.) Frémy, *op. cit.*, 1930, p. 147, Fig. 126; Frémy, *op. cit.*, 1934, Pl. 23, Fig. 2.

Lat. cell., 1·8–2·2 μ; long. cell., 4–6·4 μ.

Habitat:—Vizagapatam: on a big rock extending into the sea from the shore.

54. *Phormidium Retzii* (Ag.) Gom. Geitler, *op. cit.*, 1930–32, p. 1012, Figs. 647 *a–d*.

Lat. cell., 4·9–6·6 μ; long. cell., 4–6·6 μ.

Habitat:—Polamur: in a pond along with *Oscillatoria anguina*, *Chara* sp. and *Elodea* sp.

55. *Phormidium corium* Gom. Tilden, *op. cit.,* 1910, Pl. IV, Figs. 71 and 72.

Lat. fil., 3·6–4·8 μ; lat. cell., 2·8–3·2 μ; long cell., 3·2–8 μ.

Habitat—Narsapur: on a damp chunam wall in the writer's house; Palacol on moist bricks of a compound wall.

56. *Phormidium autumnale* (Ag.) Gom. Geitler, *op. cit.,* 1932, p. 1026, Fig. 84.

Lat. cell., 4·8–5 μ; long. cell., 2–4·8 μ.

Habitat:—Narsapur: on mud coated rocks near a water pumping engine.

Genus *Lyngbya* Agardh

57. *Lyngbya gracilis* Rabenh. Frémy, *op. cit.,* 1934, Pl. 26, Fig. 3.

Lat. fil., 8–9·9 μ; lat. cell., 5·6–7 μ; long. cell., 2–6 μ.

Habitat:—Polamur: on steps submerged in water of a pond, along with *Phormidium cebennense.*

58. *Lyngbya Nordgardhii* Wille. Geitler, *op. cit.,* 1930–32, p. 1040, Fig. 658 *a–c.*

Lat. fil., 3–4 μ; lat. cell., ·1·8–2·4 μ; long. cell., 1–2 μ; crass. vag., 0·2 μ.

Habitat:—Namdur: epiphytic on *Lyngbya majuscula* var. *chakiaense* in a pond.

In this form the constrictions at the joints are not so prominent as in the type.

59. *Lyngbya arboricola* Brühl et Biswas. Brühl et Biswas, Commentationes Algologicae ii. Algae epiphyticae epiphloiae indicae or Indian Bark Algae, *Journal of the Department of Science,* Calcutta University, 5, 1923, Pl. III, Fig. 10 *a–e;* Geitler, *op. cit.,* 1930–32, p. 1053.

Lat. fil., 19·8–26 μ; lat. trich., 16·5–17·5 μ; long. cell., 4·8–6·6 (–10) μ; crass. vag., upto 4 μ.

Habitat:—Ellore: on the bark of *Mangifera indica.*

60. *Lyngbya ceylanica* Wille. Geitler, *op. cit.,* 1930–32, p. 1055, Fig. 668 *a;* Ghose, On a collection of Myxophyceae from Mergui and some neighbouring islands, *Journal of the Burma Research Society,* 1927, Vol. XVII, Part III, pp. 224–251, Pl. III, Fig. 11.

Forma.

Lat. fil., 9·6–13·2 μ; lat. cell., 7·2–9·6 μ; long. cell., 1·6–3·2 μ; crass. vag., 1·2–2·5 μ.

Habitat:—Namdur: on moist soil amidst rice-fields, along with *Microcoleus chthonoplastes.*

The cells in this form are shorter.

61. *Lyngbya majuscula* Harv. Frémy, *op. cit.*, 1934, Pl. 28, Fig. 1 *a* and *b;* Geitler, *op. cit.*, 1930–32, p. 1060, Fig. 672 *c* and *d;* Tilden, *op. cit.*, 1910, Pl. V, Fig. 42.

Lat. fil., 43–53 μ; lat. trich., 30–39 μ; long. cell., 1·6–4 μ; crass. vag., 5–10 μ.

Habitat:—Mogaltur: on stones in the salt creaks.

Var. *chakiaens* Rao. Bhashyakarla Rao, *op. cit.*, 1936, p. 172, Fig. 3 *e–f.*

Lat. fil., 36–50 μ; lat. trich., 26–36 μ; long. cell., 4–8·2 μ; crass. vag., 4·8–5 μ.

Habitat:—Namdur: in a pond along with *Lyngbya confervoides;* Cocanada: in roadside puddles.

62. *Lyngbya confervoides* Ag. Tilden, *op. cit.*, 1910, Pl. V, Fig. 39; Frémy, *op. cit.*, 1934, Pl. 28, Fig. 2; Carter, *op. cit.*, 1933, p. 162, Fig. 11.

Lat. fil., 16·5–21·4 μ; lat. trich., 11·6–14 μ; long. cell., 2–4 μ; crass. vag., upto 4 μ.

Habitat:—Namdur: in a pond, along with *Lyngbya majuscula* var. *chakiaense;* Polamur: in a shallow well; Chittavaram: in a pond, along with *Aulosira Fritschii, A. fertilissima* var. *tenuis* and *Oscillatoria Hameli;* Namdur: in a road-side pond, along with *Tolypothrix robusta* forma and *Oscillatoria princeps.*

63. *Lyngbya semiplena* Ag. Tilden, *op. cit.*, 1910, Pl. V, Fig. 38.

Lat. fil., 13·2–17·6 μ; lat. trich., 7–10 (–11·2) μ; long. cell., 1·6–2·5 μ; crass. vag., 2·4–3·3 μ.

Habitat:—Vizagapatam: in a rocky puddle on the sea-shore.

64. *Lyngbya aerugineo-coerulea* (Kütz.) Gom. Frémy, *op. cit.*, 1930, p. 193, Fig. 157; Ghose, *op. cit.*, 1926, Pl. VI, Fig. 7.

Lat. fil., 5·6–6·4 μ; lat. cell., 4·8–5·6 μ; long. cell., 2·4–5·6 μ; crass. vag., 0·6–1 μ.

Habitat:—Palacol: in a tank (Ram Gundam) along with *Oedogonium* sp.; Cocanada: on the cemented area near a house tap, (J. Venkateswarlu).

65. *Lyngbya nigra* Ag. Geitler, *op. cit.*, 1930–32, p. 1063, Fig. 675 *a.*

Lat. fil., 10–13·2 μ; lat. trich., 7–10 μ; long. cell., 1·8–3·3 μ; crass. vag., 1–1·5 μ.

Habitat:—Cocanada: in a road-side puddle.

66. *Lyngbya corbieri* Frémy.   Geitler, *op. cit.,* 1930–32, p. 1065, Fig. 678.

Lat. fil., 10·4–12·8 μ; lat. trich., 7–9·9 μ; long. cell., 3·2–5·6 μ; crass. vag., 1·5–3 μ.

Habitat:—Vizagapatam: in a rocky puddle on the hillock extending into the sea from the shore.

67. *Lyngbya major* Menegh.   Tilden *op. cit.,* 1910, Pl. V, Fig. 46; Geitler, *op. cit.,* 1930–32, p. 1066, Fig. 679 *a;* West, *op. cit.,* 1916, p. 42, Fig. 28 *A.*

Lat. fil., 20–26 μ; lat. trich., 13–17·6 μ; long. cell., 1·6–3·3 μ; crass. vag., 3·5–4·5 μ.

Habitat:—Cocanada: on mud and stones submerged in water of a puddle, along with *Lyngbya nigra.*

Genus *Microcoleus* Desmazieres

68. *Microcoleus chthonoplastes* Thuret.   Geitler, *op. cit.,* 1930–32, p. 1134, Fig. 739.

Diam. vag., 13–105 μ; lat. vag., 3–30 μ; lat. cell., 3·2–4 μ; long. cell., 3–8 μ.

Habitat:—Narsapur: on water-logged soil in a rice-field; Namdur: on moist soil amidst rice-fields, along with *Lyngbya ceylanica* forma; Cocanada: on moist soil near the Botanical Laboratory of Pithapur Rajah's College.

This investigation was carried out in the Botanical Laboratory of the Benares Hindu University while the writer was a Research Scholar there.   The writer takes this opportunity to express his great indebtedness to Professor Y. Bhâradwâja for his kind criticism and advice during the preparation of this paper.

PARTHENOCARPY IN *DODONEA VISCOSA*

BY

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Dodonea viscosa Linn. (Family Sapindaceae) is widely distributed all over India. Being a hardy ever-green shrub, it is commonly grown for hedges. The plants for this purpose are raised from seeds, which are formed inside generally 3-winged septicidal capsules. The flowers are mostly dioecious, rarely monoecious or polygamous. The seeds are formed when both male and female plants grow together and pollination takes place easily.

My attention was drawn towards the occurrence of parthenocarpy in this species by an accident. I had a number of these plants growing in the compound of my house. Gradually, however, most of them died as a result of attacks by white ants, so that last year only one plant was left. In the last February I saw this plant flowering. Only female flowers were produced. Yet I found most of them developing into fruits. To be further sure that this was not at all due to pollination or if it was or not the effect of foreign pollen, I bagged a large number of flower buds. Even then I found many of them developing into fruits (Fig. 1). Dissection of these fruits showed that there were no seeds inside. The ovules were found to have degenerated owing to the absence of fertilisation (Fig. 2). It is thus quite clear that the gynoecium of *Dodonea viscosa* has the power of developing parthenocarpially in the absence of pollination and without the action of any other external stimulus.

The parthenocarpic fruits are just like the normal fruits except that they contain no seeds. They reach the same size and form. The only difference visible externally is in the behaviour of the style and the stigmas. The style remains persistent and green for a much longer time than in normal fruits. It begins to dry only when the whole fruit begins to dry, and even grows in length as the fruit develops. The stigmas in the parthenocarpic fruits remain unclosed till the end.

A list of known cases of parthenocarpy would lead one to believe that the phenomenon is mostly confined to the cultivated fruit plants. Its occurrence in such wild plants as *Tamarix dioica* (Joshi and Kajale, 3) and *Dodonea viscosa*, however, shows that it is likely to be found wide-spread in nature also, when a proper investigation is made of these plants.

About the cause of such growth, it is not desired to say much just at present. Since 1902, when Massart (4) placed dead pollen on the stigma of an orchid and observed slight enlargement of the ovary, there has been increasing evidence to believe that parthenocarpy results from the action of foreign pollen. The recent works of Yasuda and his collaborators (5 and 6) and Gustafson (2) provide good illustrations of this view. From a large number of experiments, the latter author concludes that there is present in the pollen of some plants a substance (or substances) which initiates growth of ovaries and in some instances causes seedless fruits to be formed. Gustafson (1) has also caused fruits to develop parthenocarpially by treating the pistils with indole-3n-propionic, indole-acetic, indole-butyric and phenyl-acetic acids. Mechanical injury has also been found in some instances to bring about changes that normally occur only when pollination and fertilisation are consummated.
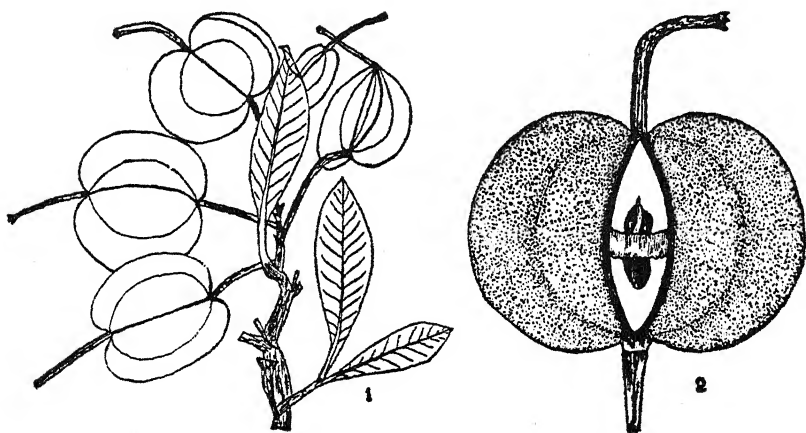
Figs. 1—2. *Dodonea viscosa.*

Fig. 1.—A small twig which was bagged before any of the flowers had opened and now shows a bunch of 5 parthenocarpially developed fruits. × 1¼. Fig. 2.—A parthenocarpic fruit with one of the loculi opened by removing one of the wings, showing the persistent style, unclosed stigmas and the degenerating ovules. × 2½.

The pistils of *Dodonea viscosa* and *Tamarix dioica*, however, have the property of developing parthenocarpially without the action of any external stimulus. One may, therefore, venture to suggest that the carpels of some flowering plants have the inherent capacity of growing to their full stature (*i.e.*, the fruiting condition) without any regard for pollination or development of seeds. That this should happen, in a way, is not at all surprising. The carpels are after all sporophylls. Among the ferns and other Pteridophyta,

the sporophylls grow to their full size by the time of spore forma-
tion. The formation of the next sporophytic generation, it being
quite independent of the parent sporophyte, has no controlling
effect on the size and form of the sporophylls of the parent
sporophyte. That the same should happen in some of the flowering
plants like *Dodonea viscosa* is perhaps just a remnant of this phase.
In most of them the full growth of the carpels has become
dependent on pollination, fertilisation and subsequent development
of the next sporophytic generation. In a few forms perhaps it is
not so dependent.

Summary

Pistils of *Dodonea viscosa* develop into parthenocarpic fruits
when pollination is prevented. Such fruits are quite similar to
normal fruits except that they contain no seeds and differ slightly
in the behaviour of the style and stigmas. No external stimulus is
necessary for such development and it is suggested that the carpels
of some flowering plants have the inherent capacity of growing to
their full stature (the fruiting stage) without any regard for polli-
nation or development of seeds.

Literature Cited

1. GUSTAFSON, F. G. Inducement of fruit development by
growth-promoting substances. Proc. Nat. Acad. Sci.,
22: 628-636, 1936.

2. ————Parthenocarpy induced by pollen extracts. Amer.
Jour. Bot., 24: 102-107, 1937.

3. JOSHI, A.C. and L. B. KAJALE. A note on the structure and
development of the embryo-sac, ovule and fruit
of *Tamarix dioica* Roxb. Ann. Bot., 50: 421-426,
1936.

4. MASSART, J. Sur la pollination sans fecondation. Bull. Jard.
Bot. de l'état, Bruxelles, 1: fasc. 3, 89-95, 1902.

5. YASUDA, S. Parthenocarpy caused by the stimulus of pollina-
tion in some plants of Solanaceae. (Japanese with
English résumé). Agriculture and Horticulture, 9:
647-656, 1934.

6. YASUDA, S., T. INABA and Y. TAKAHASHI. Parthenocarpy
caused by the stimulus of pollination in some plants
of the Cucurbitaceae. (Japanese with English
résumé). Agriculture and Horticulture, 10: 1385-
1390, 1935.
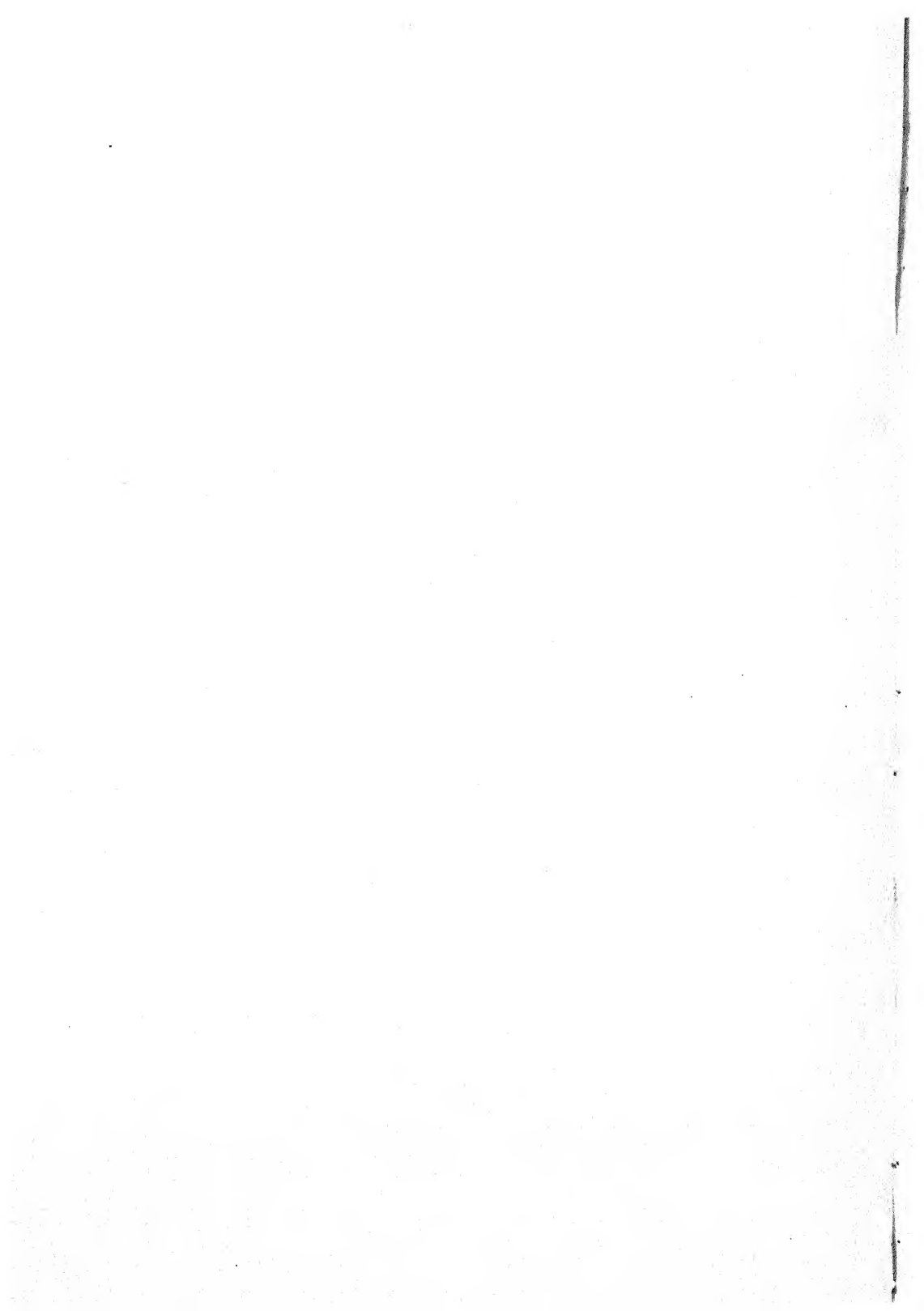
GAMETOGENESIS AND EMBRYOGENY IN SOME COMMELINACEAE *

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Communicated by *M. A. Sampathkumaran*

Contents

1. Introduction

Among the Commelinaceae, considerable work has been done only in the genus *Tradescantia*. Cytologists have always promptly turned to the large chromosomes of species of *Tradescantia* as to those of the Liliaceous plants to verify their interpretations of

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the structure and behaviour of the chromosomes in general. But it is only recently that species of certain other genera have been investigated even from the cytological point of view. Hance (1915) worked out the pollen development in *Zebrina pendula*. The characteristic formation of tapetal periplasmodium in the anthers of the Commelinaceae has successively attracted the attention of Tischler (1915), Mascré (1925), and Stenar (1925). A critical study of multiple ring formation and other evidences of 'structural hybridity' in some representatives of the section Tradescantieae has been made by Darlington (1929). Rau (1930) has described certain interesting features like the leptotene- and pachytene-bouquets, pseudo-reduction of the number of chromosomes, diakinetic deconjugation and the diffuse stage in the pollen mother cells of *Cyanotis cristata* Schlt.

The monocotyledons exhibit great variability in the details of their life-history as compared with the dicotyledons. Though considerable work has been done in the other monocotyledonous families still there is much scope for detailed investigations of the Commelinaceous plants from this point of view. Strasburger (1879) described the normal mode of development of the embryo sac in *Tradescantia virginica*. According to Stenar (1925) the same holds good for the embryo sac of *Tradescantia albiflora* also. It is, however, worth noting that in *Commelina stricta*, Guignard (1882) found the *Scilla* type of development of the female gametophyte, wherein the outer one of only two megaspores functions, whereas Maheshwari and Singh (1934) have observed the normal linear tetrad of megaspores in *Commelina benghalensis*. But considerable interest attaches to the embryo of the Commelinaceae. Solms-Laubach (1878) stated that in the two genera, *Tinnantia* and *Heteractia*, no suspensor is developed as in the Pistia type, and that the stemtip is terminal in origin but is later forced to one side by the strong growth of the cotyledon, which is here of lateral origin. Later on, Suessenguth (1921) worked on the embryo of *Tradescantia myrtifolia* but left certain important points unsettled.

2. Materials and methods

Unlike in *Commelina benghalensis*, none of the plants here selected for study produces cleistogamous flowers.

Fixations were carried out on bright days at different hours. Divisional figures were best obtained between 1 p.m. and 3 p.m. Several fixatives were tried. The best results for all stages were, however, obtained with Bouin's fluid. Since in the study of the embryo difficulty in cutting was anticipated, the material was cleared in cedar oil instead of in xylol. Still much trouble was felt in securing sections of the seed showing advanced stages of the embryo on account of the thick-walled seed coat.

Sections were cut from 6 microns to 12 microns in thickness. They were satisfactorily stained in Haidenhain's iron alum haematoxylin.

3. Megasporogenesis and the development of the female gametophyte

(*a*) *Cyanotis cristata* Schlt.

Two ovules develop in each locule of the trilocular ovary. The ovules are axile and orthrotropous and are situated one above the other with their micropyles in opposite directions.

The nucellus appears as a mass of cells protruding into the locule from the axile placenta. A ring of cells arises below the apical portion of this nucellar mass, forming the first integument. A similar ring of cells is formed lower down and constitutes the second integument. The inner integument is uniformly composed of two layers of cells, the outer integument is more massive.

The single hypodermal megaspore-mother-cell can be recognised even before the integuments arise. During the early meiotic prophase in the nucleus of this cell, a 'thin bouquet' is formed by the polarisation of the leptotene threads towards the nucleolus. During the second division, the chalazal daughter cell divides first into two. Later on, however, the micropylar daughter cell divides, completing the formation of the tetrad of megaspores. The spindle of this division is sometimes oblique, when the two megaspores that result are separated by a diagonal wall. (Figs. 1 & 2, Pl.)

The female gametophyte is developed from the chalazal megaspore. No distinct cap is formed by the disorganized megaspores, but corresponding to them in position are found three irregularly distributed black masses. Two successive divisions of the nucleus of the functional megaspore take place leading to the formation of the binucleate and the tetranucleate embryo sacs. Next, in the early eight nucleate stage, it is of interest to notice the comparatively large size of the two polars which later fuse in the centre of the sac. The antipodals are small and ephemeral. In shape, the mature embryo sac is linear and narrow at its antipodal end. (Figs. 3, 4 & 5.)

Certain modifications appear in the ovule after the octonucleate embryo sac is formed. The ovule grows very much in size. A collar-like constriction arises below the micropylar region, and divides the ovule into an upper, smaller, dome-like part, and a bigger lower part which contains a large mass of nucellar tissue. Humphrey (1896) has noticed a similar structure of the ovule in the Scitamineae. He calls the constriction the 'micropylar collar' and thinks that it facilitates the supply of nutrition to the developing embryo. The mature embryo sac of *Cyanotis cristata* is restricted to the domelike portion of the ovule. But soon after fertilisation, the embryo sac begins to penetrate into the nucellar tissue below by the elongation of the antipodal extremity of the sac. The primary endosperm nucleus divides earlier than the fertilised egg, and some of the first formed endosperm nuclei occupy the lower region of the embryo sac (Fig. 10).

The tissue near the chalazal region of the ovule is packed with some intensely staining cell contents. The same sort of cell contents are found in the inner layer of the inner integument, which, however, loses them abruptly beyond the region of the micropylar collar. At this region, these cells of the inner integumentary layer are much distended with these contents. While the ovule increases in size after fertilisation, there appear four infoldings of the wall, which develop into the pits found on the seed.

(b) *Ancilema spiratum* R. Br.

The trilocular ovary contains one or two rows of orthrotropous ovules in each locule. The number is much greater than in either *Cyanotis* or *Zebrina*.

The archesporial cell is hypodermal in origin and cuts off a parietal cell, which later divides by a vertical wall. These parietal cells persist for varying lengths of time, either dwindling away very early or still being recognisable when the four-nucleate embryo sac is formed.

During the second division in megasporogenesis, the two spindles in the daughter cells are directed at right angles to each other. A T-formed arrangement results. It is the chalazal megaspore that develops into the embryo sac (fig. 8).

An eight nucleate embryo-sac is formed as usual. Polar fusion then takes place and the antipodals are so ephemeral as to be difficult to detect. The mature embryo-sac is not narrow in the antipodal end. Though the micropylar portion of the ovule is narrow, still it is not delimited by a definite micropylar collar as in *Cyanotis*. The chalazal region and a small extent of the inner integument are, however, full of some prominent cell contents.

(c) *Zebrina pendula* Schn.

The hypodermal archesporial cell cuts off a single parietal cell. The megaspore mother cell enlarges in size and undergoes the usual heterotypic division. It was reported by the author (1934) that after the second division no wall is formed between the two upper megaspore nuclei, but subsequent observations have revealed the existence of a faint wall.

No regular cap is formed by the non-functional megaspores. The chalazal megaspore develops into the embryo-sac in the usual way. The narrow hyaline portion of each synergid shows the appearance of a filiform apparatus (Fig. 9). The antipodals disorganise and the mature embryo-sac is not narrow in the antipodal end.

4. Microsporogenesis

(a) *Cyanotis axillaris* R. & S.

The microsporangium.—There are two wall layers between the epidermis and the tapetum. The outer forms the endothecium; the inner becomes flattened and crushed, and finally disorganises.

The tapetum at first consists of tabular uninucleate cells, whose walls disorganise by the time synizesis is reached in the microspore mother cells. The protoplasts unite to form a true periplasmodium. In the early stages of its formation, mitotic divisions take place in the tapetal nuclei (Figs. 20 and 21).

Sporogenesis.—Before entering into the meiotic prophase, the pollen mother cells are polygonal in shape and closely packed together. In the resting condition the nucleus of the microspore mother cell shows a faintly staining, large-meshed reticulum of delicate threads, and it is only where two or more of these threads cross that small aggregations of chromatin occur. No prochromosomes are to be observed. There are one or two nucleoli (Fig. 11).

The onset of the heterotypic prophase is marked by a gradual contraction of the reticulum away from the nuclear membrane on all sides. Faint thin threads connect the central contracting mass of chromatin with the nuclear membrane (Fig. 12). The pollen mother cells now gradually separate from one another and become spherical in shape. A tight knot of chromatin is organised by the contracted mass, now placed on one side of the nuclear cavity. The nucleolus is not included in the knot but projects on one side. All connections with the nuclear membrane break down.

A gradual unloosening of the knot follows. The open spireme stage shows large loops spread out in the nuclear cavity, and these loops twist around one another in certain places. Splits are observed in small bits of the thick thread which presents a twisted appearance. Definite connections of the chromatin with the nucleolus are absent.

The loops are then gradually drawn towards the centre or they are irregularly contracted to a side of the nuclear cavity. The resulting second contraction figure is a densely staining, tight knot with an irregular shape, resembling the same stage of *Oenothera* (Cleland, 1926). A connection of the nucleolus with the chromatin is often present. But no changes in the staining capacity of the nucleolus were observed during the whole course of the prophase.

At the end of the second contraction stage, very thick and short loops are seen connected by one end at the centre of the knot. These break away or separate from the knot at their basal ends and gradually condense into the bivalents or gemini. During the diakinesis itself many of the bivalents assume the Y-form. Faint threads are found attached to the ends of the gemini. Even at this stage the nucleolus stains intensely.

The nuclear membrane is then resorbed, and the gemini are grouped together, exhibiting the condition of third contraction (Nothnagel, 1916). The achromatic figure arises in the cytoplasm in several places but soon becomes bipolar. During the prophase a perinuclear zone is found as in *Gossypium* (Denham, 1924).

Polar views of the metaphase show ten bivalents of varying sizes. Lagging is often met with in the anaphase and each

chromosome shows a marked bend in the middle (Figs. 13, 14 and 15).

At the end of the telophase a definite cell plate appears and cytokinesis results in dyads. During the homeotypic division, the two spindles may be found either parallel to or at right angles to each other. Cytokinesis again takes place by the cell plate method. This successive bipartition of the pollen mother cell results in the isobilateral arrangement of the microspores, but the tetrahedral arrangement is also met with occasionally.

(b) Aneilema spiratum R. Br.

The microsporangium.—The structure of the microsporangium is the same as in *Cyanotis.* A true tapetal periplasmodium is formed here also.

Sporogenesis.—The resting nucleus of the pollen mother cell reveals many faintly staining chromatin aggregations on a close-meshed reticulum. There are usually two nucleoli and nucleolar budding is common.

As in *Cyanotis axillaris,* there is a direct contraction of the chromatin reticulum into the synizetic knot. On recovery from the first contraction, the chromatin thread is thick but not very uniform. There is a clear indication of its double nature.

During early diakinesis the total number of distinct chromatin bodies observed is often more than the haploid number of chromosomes in this plant, namely, twenty. But when the nuclear membrane is resorbed preparatory to spindle formation, only twenty definite bivalents are found in the pollen mother cells. On account of the small size of the chromosomes, whether fragmentation of certain of the bivalents takes place or whether there is a temporary separation of the univalents could not be ascertained (Figs. 17 and 18).

At the end of diakinesis, the gemini remain irregularly scattered. Multipolar spindle fibres now appear but soon become bipolar. Twenty bivalents can be counted in polar views of the metaphase and they are of different sizes. Towards the late telophase a distinct cell plate is laid down, and cytokinesis results in dyads. The homeotypic division follows and isobilaterally arranged tetrads of microspores are formed. But some of the microspores are also arranged in the tetrahedral manner.

5. The male gametophyte

In *Cyanotis cristata,* of the two daughter nuclei formed by the division of the microspore nucleus, the generative nucleus soon assumes the form of a slender, curved rod. It is in this binucleate condition that the pollen grains are shed (Fig. 16).

Some pollen tubes were found in the stylar canal and also in the cavity of the ovary. The generative nucleus divides giving rise to two lens-shaped male nuclei. The pollen tube when about to pierce the embryo-sac, bears at its end a darkly stained mass representing the disorganised tube nucleus, and further inward are the two male nuclei, of which one is clearly shown in the figure (Fig. 6).

In *Aneilema spiratum* the generative nucleus is at first lenticular in shape. Later on the tube nucleus disorganises leaving a small dark patch close to the generative nucleus. The latter does not become slender and rod shaped as in *Cyanotis cristata* (Fig. 19).

6. Fertilisation in *Cyanotis cristata* Schlt.

One of the synergids is destroyed by the entering pollen tube and the two male nuclei are liberated into the embryo-sac. During syngamy, the male nucleus shows the prophasic condition, while the egg nucleus is in the resting stage. When syngamy has just taken place, the loosening spireme contributed by the male nucleus is clearly visible on one side of the fertilised egg nucleus, the rest of which still shows the resting condition (Fig. 7). In the triple fusion, the male nucleus is in the prophase, while only a faint reticulum is observed in the polar fusion nucleus. The same state of the nuclei has been reported in *Lilium philadelphicum* and *Lilium longiflorum* (Weniger, 1918).

7. Embryogeny in *Cyanotis cristata* Schlt.

The first division of the fertilised egg is transverse. Of the two cells that result, the proximal cell divides by a vertical wall. The terminal cell also divides by a vertical wall, thus forming the quadrant stage. Further divisions then take place in various planes, until a spherical mass of cells is produced. There is no definite basal cell or suspensor, all the cells formed from the fertilised egg constituting the true embryo. The apical portion of the embryo then begins to form a fairly thick circular outgrowth in the periphery. This represents the 'sheath' of the cotyledon. Soon, however, the 'lamina' of the cotyledon arises as a prominent lateral outgrowth of the 'sheath'. The lamina later gradually extends its growth all round but not at a uniform rate, so that one of its lateral margins becomes more conspicuous than the other. The sheath of the cotyledon is now not distinguishable from its lamina, the latter having merged in its growth with the former. A pore is left towards one side of the embryo by the close approach of the margins of the cotyledon, which then meet and practically obliterate the opening. By this time, the stem apex, which originates in the central depression of the apical portion of the embryo, will have assumed the form of a mound of cells. The cotyledon in this way forms a complete cover over the undifferentiated stem apex in the mature embryo. The proximal portion of the embryo becomes

very broad and disc shaped, with a characteristic rim or shelf of tissue all round. The radicle originates in the central region of this end. The terminal end of the cotyledon which encloses the "mound of cells" (the vegetative apex) broadens and also increases in thickness. An interesting feature is that the mature embryo completely fits into the cavity of the very small dome separated from the rest of the seed by the micropylar collar described before. A little cap which becomes pushed out on germination is developed at the point opposite the radicle.
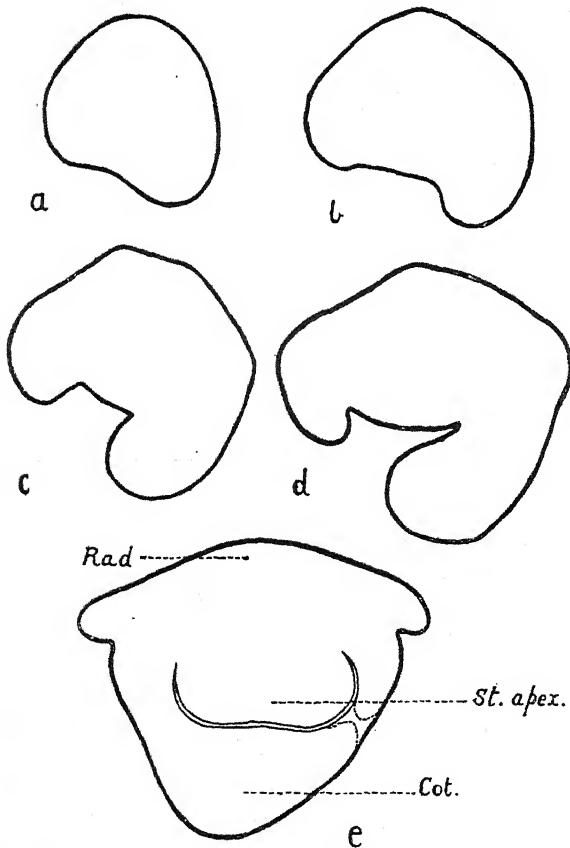
Text-fig. 1. Longitudinal sections of the embryo of *Cyanotis cristata* showing various stages of development. *a, b, c, d,* × 800; *e,* × 400.

The germination of the seed is essentially similar to that of the Date *(Phoenix dactylifera)*. But the cotyledon in *Cyanotis* becomes a spherical structure retained within the seed, with a long threadlike stalk connecting the seedling with the seed. Haberlandt

(1928) states, "Apart from the grasses, a highly organised absorbing tissue has been described only in the seedlings of Commelinaceae. In *Tradescantia erecta* the knob-shaped end of the filiform cotyledonary stalk remains embedded in the seed. This haustorial organ, which is about the size of a pin's head, is covered on all sides by a layer of specialized absorbing elements." (Text-fig. 2).

8. Discussion

(a) *Megasporogenesis and Female Gametophyte*

The development of the female gametophyte has been hitherto worked out in only a few Commelinaceous plants. Guignard (1882) has described the *Scilla*-type of development in *Commelina stricta*. In Scilla the micropylar cell of a dyad develops into the embryo-sac. But recently Maheshwari and Singh (1934) have reported a normal linear tetrad in *Commelina benghalensis*. Both in *Tradescantia virginica* (Strasburger, 1879) and in *Tradescantia albiflora* (Stenar, 1925) the female gametophyte develops in the normal way.

A T-shaped arrangement of the megaspores has been observed in *Aneilema spiratum*. This arrangement of the megaspores has been observed so far in more than forty species of Angiosperms (Schnarf, 1929).

In *Commelina stricta*, according to Guignard (1882), the antipodals are three large cells. But in all the plants studied by the writer, the general tendency is towards the early disappearance of the antipodals. A feature observed in the post fertilisation embryosac of *Cyanotis cristata* is the deep penetration by the elongation of the narrow antipodal end into the nucellar tissue below. So far as the writer is aware, this has not been observed before in the Commelinaceae. Among the monocotyledons, the penetration of the antipodal extremity of the embryo-sac for purposes of nutrition, is recorded among the Gramineae, Liliaceae and Scytamineae (Coulter and Chamberlain, 1903).

(b) *Microsporogenesis*

No chromocenters such as those observed by Rau (1930) are met with either in *Cyanotis axillaris* or in *Aneilema spiratum*.

A 'thin Bouquet' already described by Rau (1930) in the microspore mother cell of *Cyanotis cristata*, was also met with in the megaspore mother cell of the same plant. This polarisation of leptotene threads has been reported in *Osmunda, Tradescantia* and *Rumex* (Darlington, 1932). Polarisation has been regarded as an adaptation to secure regularity in pairing and the clearest evidence of parasynapsis has been found in such cases.

Among the Commelinaceae, the parasynaptic interpretation of chromosome pairing has been given by Miyake (1905) for *Tradescantia virginica*. Farmer and Shove (1905) on the other hand have advocated the telosynaptic view for the same plant and

they are supported by Mottier (1907). A clear case of parasynapsis has been described by Rau (1930) in *Cyanotis cristata*. The same interpretation holds for *Cyanotis axillaris* and *Aneilema spiratum* also, though it is not so evident here. Multiple ring formation such as is found in the classic case of *Oenothera* is also met with among the Commelinaceae in *Rhoeo discolor* where a ring-of-twelve is found arranged disjunctionally (Darlington, 1929). Such observations have often been taken to be definite proofs of the existence of telosynapsis. But recent studies have shown that the individual chromosome is not the unit of homology and thus of pairing in such cases. Each chromosome is made up of two portions, and a half of one chromosome is homologous with the half of the other; and in this way a terminalised pairing of homologous segments of chromosomes takes place.
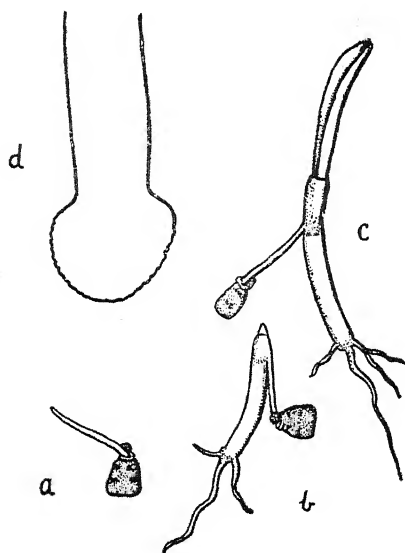
Text-fig. 2. *a, b, c.* Stages in the germination of the seed of *Cyanotis cristata.* *d,* The bulbous haustorial part of the cotyledon dissected out from the seedling of *Cyanotis cristata.*

During the diakinesis in *Aneilema spiratum*, more than the regular haploid number of chromosomes are often found. How exactly this arises is not clear. It may be due to fragmentation of some of the bivalents as in certain species of *Tradescantia* (Darlington, 1929) or to diakinetic deconjugation as described by Rau (1930) in *Cyanotis cristata*.

The achromatic spindle is formed in the multipolar fashion but soon becomes bipolar in both the plants studied, and is mostly of

cytoplasmic origin. On the other hand, Rau has described an intranuclear origin of the spindle which is bipolar from the start in *Cyanotis cristata.*

Darlington (1932) gives the diploid number of chromosomes in *Cyanotis zenonii* as sixteen, and in *Cyanotis somaliensis* as twenty-eight. In *Cyanotis cristata* the haploid number is fourteen (Rau, 1930), but in *Cyanotis axillaris* it is ten. On the view of Darlington, these different species of *Cyanotis* have evolved their respective chromosome complements by such a 'structural change' as that of fragmentation.

(c) *Periplasmodium in the anther*

Tischler (1915) and Stenar (1925) have worked out the formation of the Periplasmodium in several Commelinaceous plants. The writer has followed its development in *Cyanotis axillaris, Aneilema spiratum* and *Zebrina pendula,* in none of which, as far as the writer is aware, it had been studied before.

In the young anthers, the tapetum is at first composed of a layer of long, uninucleate cells. As pointed out by Tischler (1915), the tapetal cells lose their walls during synizesis in the pollen mother cells. The protoplasts thus liberated unite to form a nutrient plasmodium, which grows among the microspore mother cells and ramifies into the spaces left among them. The first few divisions of the nuclei of the tapetal cells are simultaneous and clearly mitotic in character, both in *Cyanotis axillaris* and *Zebrina pendula.* The occurrence of mitosis in the tapetum is not so rare as it was thought to be (Cooper, 1933). Tischler (1915) states that the nuclei of the perplasmodium of the Commelinaceae undergo changes in form and structure. The writer, however, found a rather uniform structure and only slight changes in these nuclei throughout. The peculiar rodlike bodies figured by Maheshwari and Singh (1934) in *Commelina benghalensis* are not found in the periplasmodium of the plants studied by the writer.

(d) *The Embryo*

The development of the embryo in *Cyanotis cristata* shows some important departures from the type usual among the monocotyledons. The young embryo is merely a spherical mass of cells, the suspensor being suppressed. This is the *Pistia*-type which is characteristic of the Aroideae (Campbell, 1900) (Blodgett, 1923), but is also found in the Scitamineae (Humphrey, 1896). Solms-Laubach (1878) reported it in the genera *Tinnantia* and Heterachtia. He further stated that in the Dioscoreaceae and some Commelinaceae, the cotyledon is lateral in origin rather than terminal; the stem-tip is terminal in origin but is later forced to one side by the strong growth of the cotyledon from beneath. Later on Suessenguth (1921) worked on the embryo of *Tradescantia myrtifolio,* but left the important points at issue unsettled,

Worsdell (1916) has drawn attention to the fact that in the majority of the monocotyledons, the lamina of the cotyledon greatly overreaches in development that of its sheath. He further states: "In the Dioscoreaceae and Commelinaceae, as Celakovsky points out, the state of affairs as described by Solms-Laubach is due to the fact that the sheath has developed at an earlier stage than, and consequently ahead of the lamina; the apical portion of the embryonic thallus has been used up to form the sheath, which appears in the form of a circular outgrowth; there is no shoot growing point present until a later stage; the lamina arises subsequently as a lateral outgrowth of the sheath." These remarks of Worsdell offer a good interpretation of the conditions found in the development of the embryo in *Cyanotis*. It may therefore be concluded that in the Commelinaceous embryo, both the cotyledon (sheath plus lamina) and the stem-tip are formed from the terminal or apical portion alone, the peripheral zone of which gives rise to the cotyledon whereas the central concave part forms the place of origin of the stem apex.

9. Summary

(a) *Cyanotis cristata* Schlt.

1. There is a single hypodermal archesporial cell which functions directly as the megaspore mother cell.

2. The chalazal megaspore develops into the eight-nucleate embryo-sac.

3. The embryo-sac is restricted to an upper domelike portion of the ovule formed by a circular constriction, the micropylar collar.

4. After fertilisation, the antipodal end of the embryo-sac elongates and enters considerably down into the nucellar tissue.

(b) *Aneilema spiratum* R. Br.

1. There is a single archesporial cell which cuts off a parietal cell. The latter divides into two by a vertical wall.

2. A T-shaped tetrad of megaspores is formed.

3. The embryo-sac is normal.

(c) *Zebrina pendula* Schn.

1. Only one parietal cell is cut off by the single, hypodermal, archesporial cell.

2. The chalazal megaspore forms the embryo-sac.

Microsporogenesis

1. A perinuclear zone of fibrillar nature is observed during late prophase in *Cyanotis axillaris* R. & S.

2. During diakinesis in *Aneilema spiratum*, more than the haploid number of chromosomes are often counted.

3. The haploid number of chromosomes is ten in *Cyanotis axillaris* and twenty in *Aneilema spiratum*. These have been determined for the first time.

4. In the tapetal plasmodium formed in the anthers of *Cyanotis, Aneilema* and *Zebrina*, mitotic divisions take place in the early stages.

Male Gametophyte

1. At the shedding stage, the pollen grains of *Cyanotis cristata* Schlt. contain a tube nucleus and a curved, slender generative nucleus. In *Aneilema spiratum*, the tube nucleus disorganises, and the generative nucleus is lenticular in shape.

Fertilisation

Double fertilisation was observed in *Cyanotis cristata* Schlt.

Embryo of *Cyanotis cristala* Schlt.

1. The early embryo is merely a spherical mass of cells, conforming with the Pistia-type.

2. Both the cotyledon and the stem-apex are formed by the apical (terminal) portion alone of the embryo.

3. The cotyledon forms a complete covering over the vegetative point in the mature embryo.

4. The radical is differentiated from the central portion of the broad proximal end of the embryo.

5. During germination, the knob-shaped end of the filiform cotyledonary stalk remains embedded in the seed as a haustorial organ.

Literature Cited

1. BLODGETT, F. H. (1923). The Embryo of *Lemna*. Amer. Jour. Bot. 6, 336–342.

2. CAMPBELL, D. H. (1900). Studies on the Araceae. Ann. Bot. 14; 1–25.

3. CLELAND, R. E. (1926). Cytological Studies of Meiosis in the Anthers of *Oenothera muricata*. Bot. Gaz. 82, 55–70.

4. COOPER, D. C. (1933). Nuclear divisions in the Tapetal Cells of certain Angiosperms. Amer. Jour. Bot. 20, 358–364.

5. COULTER, J. M. and CHAMBERLAIN, C. J. (1903). Morphology of Angiosperms.

6. DARLINGTON, C. D. (1929). Chromosome Behaviour and Structural Hybridity in the Tradescantieae. Jour. Genet. 21, 207–286.

7. DARLINGTON, C. D. (1932). Recent Advances in Cytology.

8. DENHAM, J. (1924). The Cytology of the Cotton Plant. Ann. Bot. 38, 407–432.

9. FARMER, J. B. and SHOVE, D. (1905). On the Structure and Development of Somatic and Heterotypic Chromosomes of *Tradescantia virginica*. Q. J. M. S. 48, 559–569.

10. *GUIGNARD L. (1882). Recherches sur le sac embryonnaire des phanérogames Angiospermes. Ann. Sci. Nat. Bot. 13, 136–199.

11. HABERLANDT, G. (1928). Physiological Plant Anatomy, p. 249.

12. *HANCE, R. T. (1915). Pollen Development and Degeneration in *Zebrina pendula*, with special reference to the chromosomes. Bull. Torr. Bot. Club 42, 63–70.

13. HUMPHREY, J. E. (1896). The Development of the seed in the Scitamineae. Ann. Bot. 10, 1–40.

14. LAKSHMINARASIMHA MURTHY, K. (1934). Gametogenesis and Embryogeny in some Commelinaceae. Curr. Sci. 4, 258.

15. MAHESHWARI, P. and BAHADUR SINGH, A. (1934). A Preliminary note on the Morphology of the Aerial and Underground Flowers of *Commelina benghalensis* Linn. Curr. Sci. 4, 158–160.

16. *MIYAKE, K. (1905). Über Reductionsteilung in den Pollenmutterzellen einiger Monocotylen. Jahr. f. wiss. Bot. XLII, 83–120.

17. *MASCRÉ (1925). Sur le Plasmodium Staminal des Commélinacees. Compt. rend. Ac. Sci. Paris 181, 1165–1166.

18. MOTTIER, D. M. (1907). The Development of the Heterotypic Chromosomes in Pollen mother cells. Ann. Bot. 21, 309–347.

19. NOTHNAGEL, M. (1916). Reduction Divisions in the Pollen mother cells of *Allium tricoccum*. Bot. Gaz. 61, 453–476.

20. RAU, N. S. (1930). On Reduction Division in the Pollen mother cells of *Cyanotis cristata*. J.I.B.S. 9, 79–113.

21. SCHNARF. K. (1929). Embryologie der Angiospermen. Berlin.

22. SCHÜRHOFF, P. N. (1926). Die Zytologie der Blutenpflanzen. Stuttgart. 453–456.

23. *SOLMS-LAUBACH, H. GRAF ZU (1878). Über monokotyle Embryonen mit scheitelbürtigem Vegetationspunkt. Bot. Zeit. 36, 65–74, 81–93.

24. *STENAR, H. (1925). Embryologische Studien. I. Zur Embryologie einiger Columniferen. II. Die Embryologie der Amaryllidaceen. Akad. Abhandl. Uppsala.

25. STRASBURGER, E. (1879). Die Angiospermen und Gymnospermen. Jena.

26. *SUESSENGUTH, K. (1921). Beiträge zur Frage des systematischen Anschlusses der Monocotylen. Beih. Bot. Centralbl. 38, 2, Abt.

27. *TISCHLER, G. (1915). Die periplasmodienbildung in den Antheren der Commelinaceen und Ausblicke auf das Verhalten der Tapetenzellen bei den übrigen Monocotylen. Jahr. wiss. Bot.. LV, 52–90.

28. WENIGER, W. (1918). Fertilization in Lilium. Bot. Gaz. 66, 259–268.

29. WORSDELL W. C. (1916). The Morphology of the Monocotyledonous Embryo and that of the Grass in particular. Ann. Bot. 30, 509–524.

* Citations from SCHNARF, K. (1929) and SCHURHOFF, P. N. (1926).

Explanation of Plates I and II

The magnifications of the figures indicated here refer to the original drawings, which have been reduced to two-thirds in reproduction, except Figs. 22 and 23 which have been reduced to half.

PLATE I

Figs. 1—10. Fig. 1. Heterotypic anaphase in megaspore mother cell of *Cyanotis cristata*. × 1800. Fig. 2. Tetrad of megaspores of *Cyanotis cristata*. × 1800. Fig. 3. Four-nucleate embryo-sac of *Cyanotis cristata*. × 1200. Fig. 4. Early stage of 8-nucleate embryo-sac of *Cyanotis cristata*. × 1800. Fig. 5. Later stage of the same. × 1200. Fig. 6. Tip of pollen tube of *Cyanotis cristata* with male nucleus in spireme condition. × 2700. Fig. 7. Syngamy in *Cyanotis cristata*. The spireme of male nucleus is still seen on one side. × 3600. Fig. 8. T-shaped tetrad of megaspores of *Aneilema spiratum*. × 1800. Fig. 9. The synergids of *Zebrina pendula* showing the 'filiform apparatus'. × 560. Fig 10. Section of the upper portion of the ovule (outer integument not shown) of *Cyanotis cristata* showing the micropylar collar and the embryo-sac after fertilization. × 560.

PLATE II

Figs. 11–21. Fig 11. Nucleus of microspore mother cell of *Cyanotis axillaris* in resting condition × 3600. Fig. 12. The same, just prior to synizesis. × 3600. Fig. 13. Metaphase of reduction division in same. × 2700. Fig. 14. Polar view of heterotypic metaphase in same with ten bivalents. × 3600. Fig. 15. Anaphase of same. Note lagging and bends in univalents. × 3600. Fig. 16. Shedding condition of pollen grain of *Cyanotis cristata* with rod-shaped generative nucleus. × 1800. Fig. 17. *Aneilema spiratum*: diakinesis showing more than twenty chromosomes. × 2700. Fig. 18. Polar view of heterotypic metaphase in same, showing twenty bivalents of varying sizes. × 3600. Fig. 19. Shedding condition of pollen grain of *Aneilema spiratum* with the degenerated tube nucleus. × 1800. Fig. 20. Mitotic divisions in tapetal plasmodium in *Cyanotis axillaris*. × 1200. Fig. 21. Tapetal plasmodium in same, later stage. × 1200.

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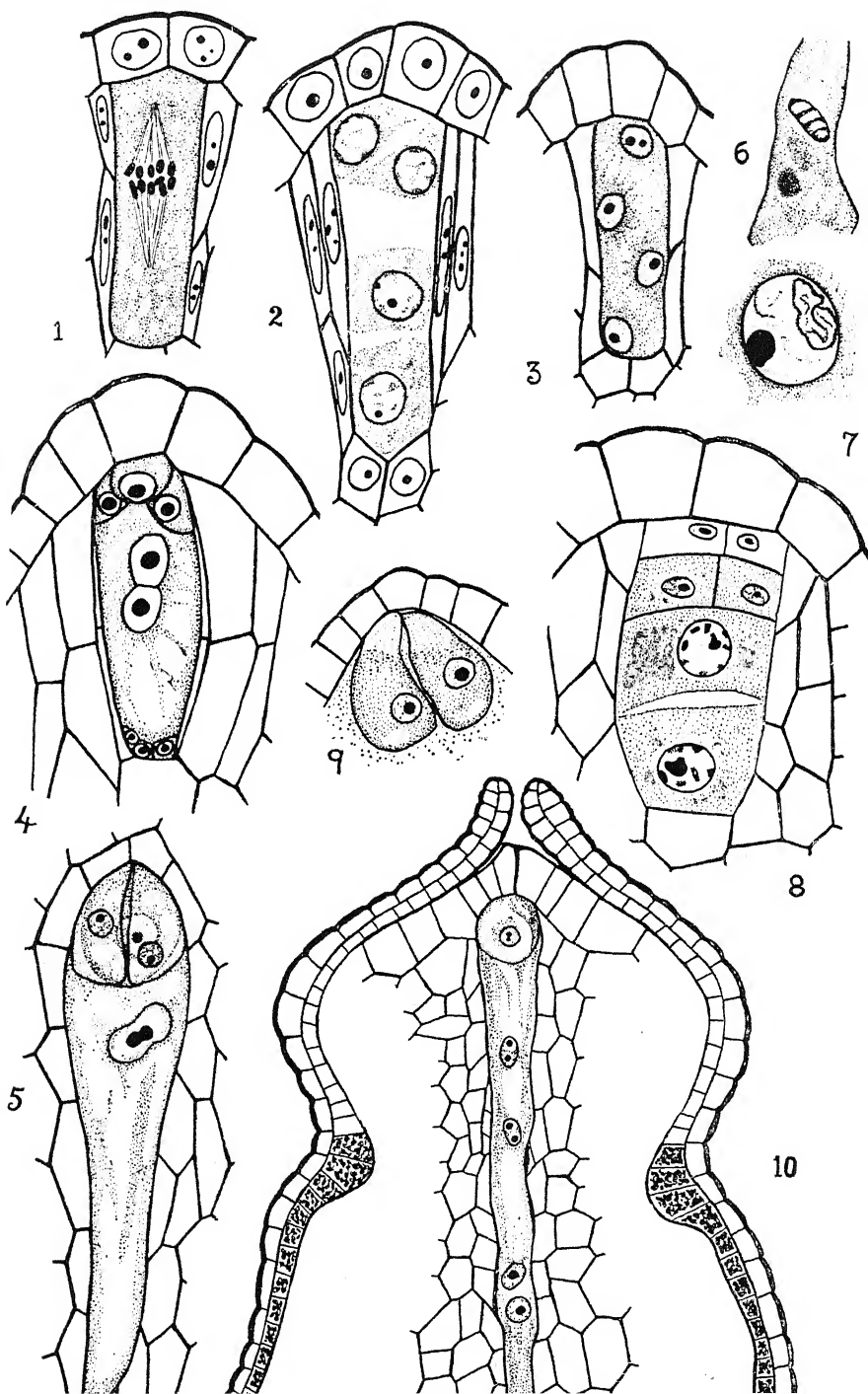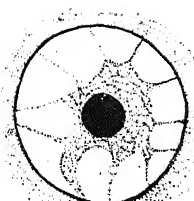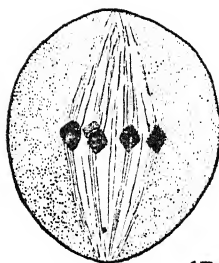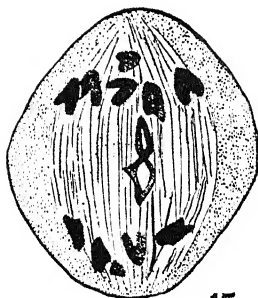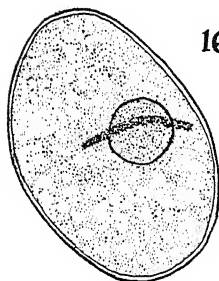
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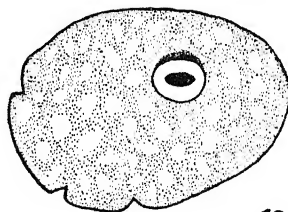
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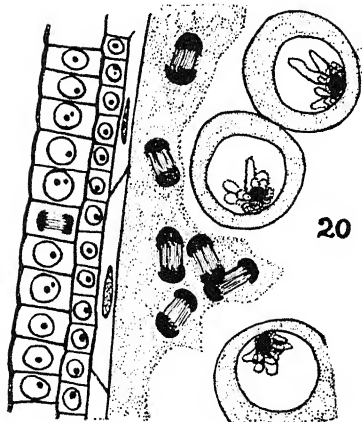
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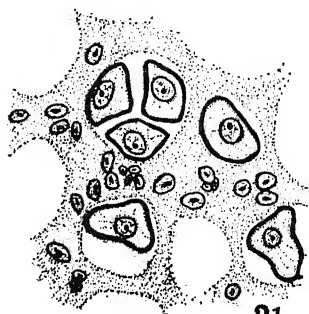
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THE MALE GAMETOPHYTE OF ANGIOSPERMS
(A CRITICAL REVIEW)

BY

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Introduction

Some recent advances in microtechnique have appreciably increased our knowledge of the male gametophyte of Angiosperms and several papers have appeared on the subject since the publication of Schnarf's "Embryologie der Angiospermen" (1929). An attempt has been made in the following pages to examine this literature[1] somewhat critically and to call attention to the places where our knowledge is still fragmentary. Another paper (Maheshwari and Wulff, 1937) describes the methods that can be used for studying the development of the male gametophyte.

In Coulter and Chamberlain's book (1903) on the "Morphology of Angiosperms," the chapter on the male gametophyte begins with the division of the microspore mother cell. We now know that the gametophytic stage with the haploid number of chromosomes commences *after* the completion of the reduction divisions. This account will therefore start with the microspore, which is the first cell of the male gametophyte, and will deal with its further history up to the time of discharge of the sperm cells in the embryo sac.

The uni-nucleate microspore

The four nuclei, formed after reduction division in the pollen mother cell, generally become separated from one another, either by furrowing or cell-plate formation, to give rise to an equal

[1] It is neither possible nor even advisable to include within this short space *all* that has been published on the male gametophyte, but every possible care has been exercised to bring together the important morphological and cytological works that have appeared since 1929. A number of older papers have also been cited, either because of their importance or the necessity of making comparisons between old and recent observations.

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number of microspores, all of which are as a rule functional. The Cyperaceae form the only important exception, for here three of the haploid nuclei degenerate and only one functions (this is the usual condition in the development of the megagametophyte). Irregularities in the reduction divisions may of course give more or less than the usual number of nuclei. The commonest cause for such abnormalities is hybridisation and this condition will not be considered further here.

The nucleus of the young microspore shows a typical resting stage, but definite information about its duration is lacking except in the case of a very few species. HEUSSER (1915) says that it is 2-3 weeks in *Himantoglossum hircinum*. In *Styrax obassia* (MANSHARD, 1936) it is less than a week and in *Tradescantia reflexa* (SAX and EDMONDS, 1933) less than four days. In *Voyria coerulea* and *Voyriella parviflora*, OEHLER (1927) reports that the nucleus never shows a real resting stage.

Division of the microspore nucleus

Before the division is to commence, the microspore nucleus comes to lie at one end of the cell while the other end is vacuolate with only a thin layer of cytoplasm along the wall[2]. GOEBEL (1933) thought that the generative cell was always cut off towards the outer wall of the tetrad, but the recent work of GEITLER (1935) shows that it is more often towards the inner side. The examples given below will serve to illustrate the range of variations that may occur:

a. *Towards outside: Helodea* (WYLIE, 1904); *Vaccinium* (SAMUELSON, 1913); *Albizzia* (MAHESHWARI, 1931); *Acacia* (NEWMAN, 1934); *Asimina* (LOCKE, 1936, judging from his figures).

b. *Towards inside: Symplocarpus* (DUGGAR, 1900); *Xyris. indica* (WEINZIEHER, 1914); *Tradescantia* (SAX and EDMONDS, 1933); *Apocynum cannabinum* (MÜLLER, 1936); *Erica, Uvularia, Narcissus, Bulbine, Gasteria, Aloe, Clivia* and several other genera of Liliaceous plants in which the pollen grains have a single furrow (GEITLER, 1935).

c. *On the radial wall: Allium* (GEITLER, 1935).

d. *In a corner[3]: Lilium* (STRASBURGER, 1908); *Anthericum, Convallaria, Ruscus* (GEITLER, 1935).

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[2] Exceptions are reported by SCHÜRHOFF (1921) in *Sambucus racemosa* and by MOHRBUTTER (1936) in *Strychnos laurina*. Here the microspore nucleus is reported to divide in the centre of the pollen grain and not near the wall.

[3] The particular angle in which the generative cell is cut off has not been determined in these cases.

GEITLER (1935) claims that the spindle has a fixed position in the microspore in every species and sometimes the whole genus and that this is uninfluenced by the mode of quadripartition of the pollen mother cell ("simultaneous" or "successive"). The place of division can of course be most easily (and also with the greatest certainty) recognised only in such cases where the pollen grains remain together in tetrads, but even when such is not the case the furrows and germ pores often prove to be thoroughly trustworthy guides. There must, however, remain a host of cases in which the pollen grains round up at a very early stage and it becomes impossible to distinguish the outer pole of the pollen grain from the inner (as in *Clarkia elegans*, GEITLER, 1937).

Among the recorded exceptions we may first refer to LAGERBERG's statement (1909) on *Adoxa moschatellina* in which the generative cell is said to be formed on any side of the wall. GEITLER's observations (1935) seem to indicate, however, that this is really not the case and that the spindle has a constant position in this species also. SAX and HUSTED (1936) report that the polarity can be disturbed by external or internal conditions and in *Periploca sepium* the generative "nucleus" may be cut off on any side although it is most often on the outer one[4]. DRAHOWZAL (1936) reports that in *Acanthus spinosus* the generative cell may be cut off towards the outer or the inner wall. JOSHI and VENKATESWARALU (1936: fig. 81) figure a peculiar case in *Nesaea myrtiflora*. Usually the pollen grains ripen free from one another in this plant, but one case was noted in which they remained together in a tetrad. Of the three pollen grains visible in the figure, two show the generative cell on the inside and one on the outside! If the figure has been correctly drawn[5], it would seem to provide a striking case of an important variation occurring in microspores produced from the same mother cell. It would be interesting to know if such a condition is of more general occurrence in *Nesaea*.

The metaphasic spindle is usually quite small and one pole is adjacent to the wall so that the resulting cells are markedly unequal in size. In a few cases like *Cotylanthera tenuis* (OEHLER, 1927), *Cuscuta reflexa* (JOHRI and NAND, 1934) and *Uvularia* (GEITLER, 1935) it extends almost through the whole width of the microspore but even here the tube and generative cell are unequal. The mechanism by which this is brought about is not quite clear. FRISENDAHL (1912) reported that in *Myricaria germanica* both ends of the spindle approximately touch the wall of the microspore but later one end contracts inwards. Whether a similar phenome-

[4] In many Asclepiadaceae the microspores show abnormal arrangements and fail to round up due to mutual pressure. This, as pointed out by the authors, accounts for the variations observed in *Periploca*.

[5] The authors themselves do not call any attention to this feature in the text, nor state anything about the usual place of division of the microspore nucleus.

tion occurs in other cases like those mentioned above, still remains to be investigated.

It is also to be noted that usually the spindle is asymmetrical with one end (that adjacent to the wall of the microspore) more blunt than the other. Thus, in the anaphase and telophase the chromosomes lie more crowded on the pole towards the centre of the grain. Exceptions are however present. Thus, in *Asclepias* (GAGER, 1902) and *Anthericum* (GEITLER, 1935) both the poles of the spindle are equally blunt and in *Adoxa* (LAGERBERG, 1909) both are pointed.

The phragmoplast also shows some interesting features, for during its increase in width the outer fibres are not directed towards the vegetative nucleus but abruptly towards the wall of the microspore, thus giving rise to the "lens-shaped" generative cell.

No case is known in which the vegetative and generative cells are equal in size. In a recent paper on some Chenopodiaceae, G. O. COOPER (1935) writes that "the microspore nucleus migrates to the periphery of the cell and there divides to form the generative and tube nuclei. By means of cell-plate formation midway between these two nuclei, two cells of *equal* size are formed." The figures, however, clearly show that the cells are unequal and this statement is perhaps due to an oversight[6].

The question whether the formation of a cell-plate in this division is also followed by a real cell-wall still remains an open one. Usually the generative cell is seen to be separated from the vegetative by a clear concave space and if a cell-wall is formed at all it must be of a very transitory nature. FINN (1935a, p. 681) clearly denies the presence of such a wall: "Auch haben wir nie eine wenn auch nur zeitweilige Bildung einer Scheidewand bei der Abtrennung der generativen Zelle im Gegensatz zu den Angaben einiger Autoren beobachtet (siehe z. B. FRIEMANN, 1910, WEFELSCHEID 1911)".

In all cases, where observations have been carefully conducted, a generative *cell* is clearly seen at least in the earlier stages. Later, during its penetration into the vegetative plasm, the nucleus alone seems to remain visible and the cytoplasm appears to have been "lost." It has, however, been shown in many cases that this is due to inadequacies of technique rather than an actual absence of the generative plasm. To cite a few such instances, PODDUBNAJA-ARNOLDI (1934) reported a naked generative nucleus in *Jasione montana*, while SAFIJOWSKA (1935) later demonstrated that it is

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[6] In exceptional cases equal cells (OLIVER, 1891; FEDORTSCHUK, 1931; MOHRBUTTER, 1936) or equal nuclei (OVERTON, 1891; SAFIJOWSKA, 1935; SAX and EDMONDS, 1935) may arise in this first division. FEDORTSCHUK and MOHRBUTTER observed, in *Cuscuta epithymum* and *Strychnos sansibariensis* respectively, that a generative nucleus was formed afterwards in one of the two daughter cells formed in this way.

a *cell*. FEDORTSCHUK (1931) reported that in *Cuscuta monogyna* the cytoplasm of the generative cell quickly disappears and only the nucleus is left, while FINN and SAFIJOWSKA (1933) showed that the generative plasm persists even up to the formation of the male cells. GURGENOWA (1928) speaks of male nuclei in *Orobanche (Phelipaea) ramosa*, while FINN and RUDENKO (1930) could trace male *cells* up to their discharge into the embryo sac (compare also WULFF, 1933, p. 25-26). In a recent paper FINN (1935a, p. 681) writes: "Auf Grund der in unserem Laboratorium ausgeführten Arbeiten kann ich bestätigen, dass bei allen Angiospermen abgesonderte generative Zellen vorkommen. Allerdings bezieht sich das nur auf die Fälle, wo die Entwicklung des männlichen Gametophyten normal verläuft und wo sich befruchtungsfähige Spermien bilden." WULFF and LINDSCHAU (1936) have also expressed a similar opinion[7].

Structure of the generative nucleus and plasm

As a rule there is a difference between the relative sizes of the generative and vegetative nuclei from the very beginning and the former can be recognised easily by its smaller size. There is no doubt that both receive an equal amount of chromatin, but as GEITLER (1935) points out, the generative nucleus receives a smaller quantity of karyolymph. That it stains more deeply than the vegetative nucleus (even with FEULGEN) may be due to this as well as the fact that it generally does not have a true resting stage.

Another long known difference in staining reaction, *i.e.*, the cyanophily of the generative nucleus and the erythrophily of the vegetative—may likewise depend on certain physico-chemical changes in chromatin which occur during the mitotic cycle rather than on the "sex" of the nuclei as was thought earlier.

The differences in the generative and vegetative plasms are also quite noteworthy in several cases, as evidenced by the use of cyanin and erythrosin, FLEMMING's triple stain, PIANEZE and even HAIDENHAIN's iron-haematoxylin. Sometimes the generative plasm does not stain at all, and TSCHERNOJAROW (1926) has good reasons to believe that: "Diese Standhaftigkeit in Bezug auf Färbung verdient ein besonderes Interesse, da ohne Zweifel die Eigenschaften eines solchen Cytoplasmas sich stark von denjenigen des gewöhnlichen unterscheiden." WULFF (1933) regarded this lack of staining capacity of the generative plasm as a sign of its beginning to disintegrate.

PIECH (1924a) expressed the view that the generative plasm consists only of proteids and does not contain any osmotically active substance. This seems to be incorrect in view of the fact that a vacuome has now been demonstrated in the generative and sperm

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[7] See also the recent paper by SUITA (1937a) who reports the presence of a "droplets-sheath" around the generative nucleus of several monocotyledons.

cells of many monocotyledons: *Haemanthus Katharinae* (Woycicki, 1926), *Clivia nobilis, Hippeastrum hybridum, Sprekelia formosissima, Scilla bifolia, Scilla amoena, Eucharis amazonica, Galanthus nivalis* and others (Wulff, 1933).

Among other inclusions in the generative plasm the discovery of chloroplasts in *Lupinus luteus, Narcissus incomparabilis,* and *Crocus vernus* (Ruhland and Wetzel, 1924) and plastids in *Lilium candidum* (Guilliermond, 1920, 1924) and *Gagea lutea* (Krupko, 1926) is specially noteworthy. Finn (1925, 1926, 1928) saw numerous minute bodies in the plasm of the generative and sperm cells of *Asclepias, Vinca* and *Vincetoxicum* but could not decide whether they were extra-nuclear chromatin granules or plastids or some other products. Rudenko (1930) noted similar bodies in the generative cell of *Lathraea squamaria* which become scattered all over the spindle when the cell divides and he thinks it likely that they are of chromatic origin. The occurrence of mitochondria in the generative plasm is suggested by Guilliermond (1920, 1924), Krupko (1926) and Anderson's figures (1936) of *Hyacinthus orientalis.* It may be remarked that the last-named author speaks of male "nuclei," while his figures as well as description show that there are *cells.*

This leads us on to the question of the occurrence of centrosomes in the male gametophyte of Angiosperms. Guignard (1891), Schaffner (1896, 1897) and some other authors figured them in various stages of gametogenesis of certain Angiosperms but Koernicke (1906) and most later workers have definitely denied their occurrence. Miss Welsford (1914), in two species of *Lilium,* and Miss West (1930) in *Viola Riviniana,* speak of some small deeply staining bodies, that suggested comparison with blepharoplasts but make no definite statement on the point. The claim that centrosomes occur in Angiosperms has been renewed by Feng (1932, 1934) who reports their presence in *Lonicera* and draws a number of figures to illustrate his point. It is, however, more likely that they are merely precipitations in vacuoles (Eichhorn, 1933) or some other bodies that have been accidentally transported to the poles. In her recent paper on the mechanism of mitosis in the pollen-tube of *Tulipa* Miss Upcott (1936) speaks of "centrosome repulsion" without giving any evidence in her figures to prove that centrosomes really exist either in the divisions in the pollen grain or pollen tube. It seems that she has used this badly chosen expression only to indicate that there are some forces supposed to act from the poles of the spindle upon the chromosomes.

Division of the generative cell in the pollen grain

The division of the generative cell usually occurs after its migration into the vegetative plasm. Sharp (1934, fig. 125) shows the dividing cell near the wall of the pollen grain which is, in our opinion, not the typical condition.

The nuclear division itself shows nothing that is essentially different from normal mitosis. In most cases the spindle appears to be intranuclear and in others it seems that both the nucleus and the cytoplasm take part in its formation (GAGER, 1902; WYLIE, 1904; LAGERBERG, 1909; PIECH, 1924b, 1928; FINN, 1925; and others).

As regards cytokinesis, WULFF (1933, pp. 39-40) gives a short review of the previous observations and concludes that it is accomplished by means of a constriction (furrow) and that phragmoplasts and cell-plates are ephemeral structures without any special function (cf. WEST, 1930 on *Viola Riviniana*, and others). On the other hand, G. O. COOPER (1935, 1936), D. C. COOPER (1935) and others have recently stressed the older view that the division is perfectly typical and a cell-plate is formed in the normal way across the mid-region of the spindle. Work of more comprehensive and comparative nature, than has been done hitherto, is necessary to elucidate this question.

Shedding stage of pollen

The pollen grains are shed at the 2- or 3-celled stage. It is difficult to say which of the two conditions is the more common one. There are more records, it is true, of binucleate pollen but there are good reasons to believe that several of these are incorrect and based on material that was not sufficiently mature at the time of fixation. In some plants both conditions occur (see list by DAHLGREN, 1916). To these may be added some others like *Iris versicolor* (SAWYER, 1917), *Elatine triandra* (FRISENDAHL, 1927), *Dionaea muscipula* (SMITH, 1929), *Viola Riviniana* (WEST, 1930), *Circaeaster agrestis* (JUNELL, 1931), *Digera arvensis* (PURI and SINGH, 1935) and *Stellaria media* (JOSHI, 1936).

WUNDERLICH (1936) observed three-celled pollen grains in some Liliaceous plants, which as a general rules possess two-celled pollen grains, but she does not make definite statements how this exceptional condition has arisen. As early as 1888, STRASBURGER showed that in *Chlorophytum Sternbergianum* a change in environmental conditions could cause the division of the generative cell to take place even in the pollen grain although it usually divides later in the pollen tube. WULFF (1934a), who found 2- as well as 3-celled pollen grains in *Impatiens parviflora* expressed the view that the latter occur in older anthers. HEITZ and RESENDE (1936), on the other hand, were unable to find any 3-celled pollen grains even in older flowers but observed that the generative cell divided (due to artificial conditions) in pollen grains that had been kept on films of sugar-agar for germination. PODDUBNAJA-ARNOLDI (1936) is similarly inclined to believe that the three-nucleate pollen grains which she observed in some of her cultures are also due to the influence of outer conditions.

In *Viscum album* (SCHÜRHOFF, 1922) the division of the generative nucleus is reported to occur while it is still inside the pollen grain, although the pollen tube has come out from one side. FINN (1928) found that in *Vinca minor* the pollen grains are generally three-celled at the time of shedding but in one case the generative cell was observed to divide on the stigma. In *Holoptelea integrifolia* (CAPOOR, 1937) the pollen grains are two-celled at the time of shedding but some three-celled ones were seen on the stigma. The author concludes that here the division occurs on the moist nutritive surface of the stigma and as a parallel cites cases like those of *Juniperus*, *Cupressus* and *Taxus* in which the first division of the microspore takes place on the nucellus before the growing out of the pollen tube.

More than three nuclei are seen only rarely and among such abnormalities the reported occurrence of a "prothallial cell" is especially interesting. CHAMBERLAIN (1897) was the first to report such a case in *Lilium tigrinum* in which sometimes a small cell is cut off before the formation of the tube and generative nuclei. SMITH (1898) in *Eichhornia crassipes,* CAMPBELL (1899) in *Sparganium simplex,* WÓYCICKI (1911) in *Yucca recurva* and FEDORTSCHUK (1931) in *Cuscuta epithymum* reported similar abnormalities. Some authors have objected to the use of the term "prothallial cell" for such cases, but recently BILLINGS (1934) saw it in a considerable number of pollen grains of *Atriplex hymenelytra* and he does not think it improbable that an Angiosperm would now and then be found exhibiting an atavistic tendency of this kind. In another member of the Centrospermales, *Stellaria media,* P. C. JOSHI (1936) has seen a four-nucleate pollen grain and he considers this fourth and smallest nucleus as homologous to the prothallial cell of *Atriplex*.

Here we may also refer to the large embryo sac-like pollen grains found by NĚMEC (1898) in the petaloid anthers of *Hyacinthus orientalis*. STOW (1930) has been able to induce their formation in normal anthers by varying the experimental conditions. Usually they have a group of three cells at each end with two nuclei fusing in the centre, but some were found to have only four nuclei (an "egg," one "antipodal cell" and two "polar nuclei") and others with sixteen nuclei (an "egg apparatus" of 5-10 cells, 1-2 "polar nuclei" and 4-5 "antipodal cells"). In a later paper (STOW, 1933) the same author shows that these "pollen-embryo sacs" simulate true embryo sacs not only in their development and structure but also show some real female tendencies of a physiological nature. One case was actually noted in which a pollen tube emerging from a normal grain of another garden variety had coiled round a pollen-embryo sac and even discharged a sperm nucleus into the latter. In still another case the "fusion nucleus" had divided into sixteen nuclei, which seems to be the consequence of its having been "fertilized" by a male nucleus, although this

point is not certain. Recently NAITHANI (1937) has also found that in the anthers from bulbs of *Hyacinthus orientalis* var. "Yellow Hammer," specially treated by breeders for early flowering, the microspore nuclei undergo three successive divisions producing embryo sac-like pollen grains.

Division of the generative cell in the pollen tube

The division of the generative cell, when it occurs in the pollen tube, is attended by some peculiarities which are evidently due to spatial conditions, *i.e.*, the narrowness of the tube.

At the time of its entry into the tube, the generative nucleus is in most plants already in a state of prophase. When the pollen tube is comparatively broad (no matter whether the observations are made on artificially grown tubes, or on anthers of cleistogamous flowers where germination occurs *in situ*, or on stylar sections) the metaphase seems to proceed normally; the chromosomes are arranged in an equatorial plate and spindle fibres are also visible (GUIGNARD, 1891, on *Lilium martagon;* LAGERBERG, 1909, on *Viola* spec.; HERRIG, 1922, on *Lilium candidum;* FRISENDAHL, 1927, on *Elatine triandra;* MADGE, 1929, on *Viola odorata* var. *praecox;* RUDENKO, 1930, on *Lathraea squamaria;* TRANKOWSKY, 1931, and WULFF, 1933, on *Hemerocallis flava;* RUDENKO, 1933, on *Verbascum lychnitis;* WULFF, 1935, on *Narthecium ossifragum;* D. C. COOPER, 1936, on *Lilium regale, L. auratum* and *L. philippinense;* MADGE, 1936, on *Hedychium Gardnerianum*). In *Elaeagnus angustifolia* FUCHS (1936) saw a normal equatorial plate but no spindle fibres. WUNDERLICH (1937) also reports absence of spindle fibres in artificially grown pollen tubes of *Muscari racemosum* and *M. comosum.*

In cases where the tubes are very narrow, spindle fibres are generally absent and instead of a regular metaphase we have a "pseudo-equatorial plate" (see WULFF, 1935). Such cases where the anaphasic separation of the chromosomes is accomplished even in the absence of the spindle fibres lend support to the view that the chromosomes have an independent power of movement. In cultures of pollen tubes of *Nemophila insignis*, WULFF and RAGHAVAN (1937) find that the division of the generative nucleus is highly irregular, for the daughter chromosomes fail to pass to the two poles and a restitution nucleus is formed.

In *Narthecium ossifragum* (WULFF, 1935) pollen tubes studied from stylar sections failed to reveal a spindle, while those grown in culture showed it quite clearly. This is evidently related to the width of the tubes which is much greater in the latter case.

With regard to the mechanism of cell-division it may be stated that in most cases neither phragmoplasts nor cell-plates have been seen (*Aconitum napellus*, OSTERWALDER, 1898; *Lilium Martagon, L. auratum*, WELSFORD, 1914; *Echeveria Desmetiana*, HERRIG,

1919; *Haemanthus Katharinae*, WOYCICKI, 1926; *Scrophularia nodosa, S. alata*, RUDENKO, 1929; *Orobanche* spp., FINN and RUDENKO, 1930; *Convallaria majalis*, TRANKOWSKY, 1931; *Lilium regale*, O'MARA, 1933; *Impatiens Holstii, Lilium Martagon, L. candidum, Hemerocallis flava*, WULFF, 1933; *Jasione montana, Campanula persicifolia, C. rotundifolia, C. patula, C. servicaria*, SAFIJOWSKA, 1935). In a few cases phragmoplasts were seen, but no cell-plate appears to have been formed (*Elatine triandra*, FRIESENDAHL, 1927; *Viola odorata var. praecox*, MADGE, 1929; *Verbascum Lychnitis, Linaria vulgaris, Gratiola officinalis*, RUDENKO, 1933; *Hedychium Gardnerianum*, MADGE, 1936). It seems, therefore, that in all these plants cytokinesis occurs by means of a constriction.

KOERNICKE (1906) and STRASBURGER (1908), both working on *Lilium Martagon*, stated that a cell-plate is formed in rare cases but is very transitory. On the other hand, GUIGNARD (1891, *Lilium Martagon*), MODILEWSKI (1918, *Neottia Nidus-avis*), HERRIG (1922, *Lilium candidum*), RUDENKO (1930, *Lathraea squamaria*), D. C. COOPER (1936, *Lilium regale, L. auratum, L. philippinense*), and UPCOTT (1936, *Tulipa spp.*) believe that it is the cell-plate which brings about the division of the generative cell into the two male cells. FUCHS (1936), working on *Elaeagnus angustifolia* did not actually see either phragmoplasts or cell-plates but believes that the division occurs by this method.

Movement of the generative cell and sperm "cells" or "nuclei"

There are, in our opinion, good reasons to believe that the division of the generative cell in the pollen tube generally occurs by a process of furrowing[8] and that further investigations are necessary to decide whether the phragmoplasts or cell-plates observed in a few cases really lead to the division of the plasm or they are merely transitory appearances.

The male gametes of most organisms are capable of active movement and therefore it is of great theoretical interest to know if the generative and sperm cells of Angiosperms also have such a power or they are merely carried along passively by the streaming movements of the vegetative cytoplasm.

The question may be considered in three phases: (1) the "movement" of the generative cell from its original position near the wall of the pollen grain towards its interior; (2) its passage through the pollen tube; and (3) last of all the movement of the male gametes in the embryo sac *after* the pollen tube has discharged its contents.

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[8] BANERJI and GANGULEE (1937) have recently reported cytokonesis by furrowing in the generative cell of *Eichhornia crassipes*.

(1) DUGGAR (1900), GAGER (1902), MURBECK (1902) and LAGERBERG (1909), and others thought that the penetration of the generative cell into the vegetative cytoplasm (when both are still within the pollen grain) depended on the activity of the latter, while STRASBURGER (1908. p. 526-527) expressed the opposite view and stated that this action implied an independent movement of the generative cell itself. FRIEMANN (1910) and WEFELSCHEID (1911) took up an intermediate position and thought that the movement was due to a joint action on the part of both. The position may be best summarized in the words of DAHLGREN (1916): "Die Frage zur annähernd sicheren Entscheidung zu bringen, ist natürlich kaum möglich, da die bisherigen Untersuchungen nur an toten Zellen gemacht wurden." It may, however, be said that nothing has so far been found to disprove STRASBURGER's view; indeed HÅKANSSON (1924) has once again stated that the generative cell seems to be the more active of the two and some other authors have implied the same although admittedly without giving a very close attention to the question.

(2) Coming now to the passage of the generative cell and the male gametes in the pollen tube, no serious objection was raised for a long time against STRASBURGER's view (1884, 1900, 1948) that this was accomplished passively by the streaming movements of the vegetative plasm within the tube. WÓYCICKI (1926) showed that the generative and sperm cells of some monocotyledons have a vacuome which readily stains with neutral red and therefore their passage can be followed with ease in cultures of living pollen tubes. WULFF (1933) used this method and found nothing to show that the streaming in the vegetative plasm had anything to do with the transport of the male cells. That the generative cell (and later the sperm cells produced from it) usually takes up almost the entire diameter of the tube and that the latter has several fine plasma streams running not in the same but in opposite directions to each other, make it impossible to avoid the conclusion that the *steady* passage of the nuclei towards the tip is due to their own motility, independent of the streaming.

Among other workers who have accepted an active movement on the part of the male gametes, based on observations of fixed material, may first be mentioned the name of MISS WELSFORD (1914). In her work on *Viola Riviniana* WEST (1930) argues that "the form of the whole male cell certainly indicates motility." More recently, SAFIJOWSKA (1935) and FUCHS (1936) have also expressed similar opinions.

Neither WULFF (1933) nor FUCHS (1936) have however, called attention to still another source which lends considerable support to this view. In an article entitled "Beziehungen zwischen Zellteilung und Zelltätigkeit" PETER (1930) has brought forth evidence to show that "Mitose und Zellfunktion hemmen einander;

eine in Mitose befindliche Zelle arbeitet nicht." We should there-
fore expect, that during the time the generative cell is occupied
with the tedious process of dividing to form the two male gametes,
it would stop its movement and lie in a comparatively quiescent
state. Such is actually the case as observed by WULFF (1933) in
Impatiens Holstii and FUCHS (1936) in *Elaeagnus augustifolia*.
The generative cell ceases to move during the late prophase, meta-
phase and early anaphase and lies in contact with the wall of the
pollen tube. Miss FUCHS (1936) saw this with special clearness
in *Elaeagnus*, where a "Plasmaschwanz" attaches it to the wall.
Towards the close of the anaphase, it resumes its passage down
the tube!

In a very recent paper MRS. PODDUBNAJA-ARNOLDI (1936)
has, however, raised some objections against this view, which appear
at the first sight to be of considerable importance. She made
cultures of pollen tubes of several plants and studied the effect of
different doses of X-rays upon them. As a result of this treatment
the nuclei were found to be unable to divide and she infers that
they were dead. Nevertheless they continued their downward
passage into the tube and from this it is concluded that they must
have been transported passively by the streaming plasm of the
tube. We must here state that MRS. PODDUBNAJA-ARNOLDI only
demonstrated the inability of the nuclei to divide (after X-ray
treatment) and not their death. It is quite probable that they
continued to live even after they were unable to divide[9].

(3) While pollen tubes can be easily grown in culture and
their living contents kept under observation for several hours,
this is hardly possible with embryo sacs, since the ovules have
usually to be fixed and sectioned before the contents of the embryo
sac can be made visible. Some embryo sacs can, however, be
studied without sectioning while they are still alive, and *Monotropa*
seems to be a specially favourable object. STRASBURGER (1900)
who studied *M. hypopitys* thought that the passage of the male
nuclei towards the egg cell and the polar nuclei was accomplished
passively. SHIBATA (1902) investigated another species of the same
genus, *M. uniflora,* but uses a more guarded language: "Ob die
Spermakerne eine selbständige Beweglichkeit besitzen oder nicht, bleibt
noch eine Sache der Diskussion . . . Die gekrümmte Gestalt der
Spermakern kann ebensowohl als plastisches Nachgeben, als wie
ein Zeichen activer Bewegung gedeutet werden."

After this no further observations appear to have been made
on living embryo sacs to elucidate this question, but in view of
the frequently curved or "worm-like" form of the nuclei many
authors have expressed themselves against STRASBURGER's view.
Among them we may specially cite NAWASCHIN (1909), who

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[9] As pointed out recently by SUITA (1937b), Mrs. Poddubnaja-Arnoldi's
statements seem to lead to the conclusion that the nuclei are not needed for
the life of the pollen tube! This is obviously quite incredible.

repeatedly speaks of the motility of the sperm nuclei. There are of course many instances where the sperm nuclei are round or oval and their shape does not give any suggestion of motility. NAWASCHIN (1897) and NAWASCHIN and FINN (1912) in *Juglans* and TSCHERNOJAROW (1926) in *Myosurus minimus* observed that at the time of their entrance into the embryo sac the sperm nuclei have a common sheath of generative cytoplasm, out of which they later slip out ("ausschlüpfen") and then take part in fertilization[10]. This act of freeing themselves from the generative plasm is in the words of TSCHERNOJAROW, "nicht ohne aktive Bewegung von ihrer Seite." and similar conclusions are made by GUERGENOWA (1928).

Taking everything into consideration we have good reasons to support the view expressed by WELSFORD, ISHIKAWA, WEST, WULFF, SAFIJOWSKA and FUCHS in favour of an active movement of the generative and the sperm cells in the pollen tube. The works of BOBILIOFF-PREISSER (1917) and VALKANOV (1934) show that cell nuclei in general have the power of autonomous movement and we should certainly expect this also in the case of the male nuclei in the embryo sac of the Angiosperms.

The vegetative nucleus

As the vegetative nucleus is larger than the generative and is in a typical resting stage, it generally stains less deeply than the latter, especially when Haematoxylin is used. In some cases it stains even more feebly than the cytoplasm of the pollen grain and this has led to the question of its supposed degeneration and lack of significance for the growth of the pollen tube. As early as 1879, ELFVING thought that in *Hypericum calycinum* it degenerated before the germination of the pollen grain. Since then SCHNIEWIND-THIES (1901), SHATTUCK (1905), FRISENDAHL (1912), DAHLGREN (1916), FINN (1928), PODDUBNAJA-ARNOLDI (1927, 1933, 1936), SAX and EDMONDS (1933), SAX (1935) and several other authors have reported a similar condition in a number of plants investigated by them.

It may, however, be objected that the invisibility of the vegetative nucleus may not be due to its actual disappearance but to its lack of affinity for the commonly used stains. WULFF (1933) showed for instance that when the FEULGEN method was employed the vegetative nucleus could be seen in several plants in which it was otherwise on the verge of invisibility. The whole question

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10 Fig. 48 of GORCZYNSKI'S (1929) paper on *Oxalis acetosella* indicates that the male nuclei may free themselves from their plasm even in the pollen tube. It would be interesting to know whether this behaviour is the rule or an exception (See also MISS SAWYER, 1917, on *Iris versicolor*: "The male nuclei may leave the generative cytoplasm, and were seen free in the tube"). It is quite probable that such appearances are artefacts caused by inadequacies of technique.

deserves a more careful study before we would be justified in concluding that it plays no active role in the growth of the pollen tube.[11]

A division of the vegetative nucleus has been recorded in several plants; especially interesting are the observations of SAX (1935) in *Tradescantia*, where a division could be induced by an increase of temperature. Statements on a "fragmentation" of the tube nucleus must, however, be accepted only with reservation since its irregular outline and week stainability (combined with the lack of uniformity with which the different regions respond to the commonly used stains) has often led to erroneous conclusions. To cite one instance, HERRIG (1922) reported a fragmentation of the vegetative nucleus in *Lilium candidum*, while WULFF (1933) found that this was really not the case at all.

The male gametes in the embryo sac

In general the sperm cells (or nuclei) are round or oval at the time of their discharge into the embryo sac but various exceptions have been noted and at least in the Compositae the filamentous or spirally curved form seems to be the more frequent one.

Although several investigators have seen the sperms organized as definite cells up to the time of their discharge into the embryo sac[12], no one has definitely succeeded in demonstrating that the male plasm actually enters the egg. In his paper on *Vallisneria spiralis,* WYLIE (1923, p. 196) writes: "It seems certain, however, that some or all of the sperm cytoplasm would enter the egg with the male nucleus." FINN (1935b) also says: "Obgleich es nicht gelungen ist, den Prozess des Eindringens in die Eizelle bei *Asclepias cornuti* als solchen zu beobachten, so muss man, nach dem Zustand ihres Plasmas im Embryosack zu urteilen, die Teilnahme des männlichen Plasmas an der Befruchtung bei dieser Pflanze als sehr wahrscheinlich annehmen." In *Butomopsis lanceolata,* JOHRI (1936) could trace the male gametes as *cells* up to the time of their discharge in the embryo sac but did not succeed in observing the penetration of the male cytoplasm into the egg.

It is true that certain phenomena in heredity speak in favour of the idea that the male plasm also enters into the fusion, but this

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[11]SUITA (1937b) has made a thorough study of the development of the pollen in *Crinum* and finds that even after using Feulgen's method the vegetative nucleus stains only faintly. He believes therefore that it is a degenerate element.

[12] The painstaking observations of FINN and his collaborators at Kiew (U.S.S.R.) are specially important in this connection and the series of papers published from this laboratory have demonstrated the presence of sperm *cells* in many cases where only *nuclei* were reported formerly. We think it likely that generative and sperm *cells* will be found to be of general occurrence in Angiosperms and in such families as the Compositae where only male *nuclei* (except by MERRELL, 1900), have been reported till now, the film of plasma around them is so thin as to have escaped notice.

has not yet been cytogically demonstrated. KIYOHARA (1935) has recently called attention to the possibility of even the vegetative plasm taking part in fertilization, and reports that in *Oenothera tetraptera* the spindle-shaped starch grains of the pollen tube enter the egg cell and can be traced even up to the four-celled embryo.

A few observers have called attention to a difference in size between the two sperm nuclei discharged from one pollen tube. Thus, BLACKMAN and WELSFORD (1913) on *Lilium*, SAWYER (1917) on *Iris versicolor*, HOARE (1934) on *Scilla sibirica*, and NEWMAN (1934) on *Acacia Baileyana* report that the male nucleus fusing with the polars is somewhat larger in size than the one which fertilizes the egg. NAWASCHIN (1927) and GURGENOWA (1928) stated that the nucleus destined to fertilize the egg stains less intensively than the other. PERSIDSKY (1926) writes of *Orobanche cumana* and *O. ramosa* "der die Eizelle befruchtende männliche Kern ist mach Gestalt und Grösse anders geartet, als derjenige, welcher sich mit den Polkernen vereinigt. Der erste männliche Kern ist halbkugelförmig und grösser als der letzte, der eine ovale Form hat." In *Lathraea squamaria* (RUDENKO, 1930) the nucleus that fuses with the egg is spherical while the other is more elliptical.

FINN (1928) reports that in *Vinca minor* even the sperm *cells* are unequal, one with a longer "Plasmaschwanz" than the other.

Systematic Conclusions

SCHÜRHOFF (1926) was probably the first to attribute a systematic significance to the number of nuclei in the mature pollen grain and expressed the opinion that two-celled pollen grains are to be found in the more primitive families and three-celled in the more advanced ones. The fact that she succeeded in varying the number of nuclei from 2 to 3 by altering the external conditions has led PODDUBNAJA-ARNOLDI (1936) to express a different opinion.

The presence of a clearly distinguishable sheath of cytoplasm round the generative nucleus and later the male nuclei as well as their general shape are quite characteristic of certain families. WUNDERLICH (1936) has recently made use of the structure of the generative cell to elucidate the relationships of certain genera of the Liliaceae.

FINN (1928b) reports that in *Fagus silvatica* the pollen tube is repeatedly branched and resembles a fungus mycelium. The fact that this is known to be the case in many other plants of the "Amentiferae" seems to indicate that even the behaviour of the pollen tube is not without some systematic value in narrow circles of affinity. It is obvious, however, that the male gametophyte is only one of the clues for taxonomic evaluation. A more detailed

discussion of this question will be found in a forthcoming paper by Prof. K. Schnarf, dealing with the value of the embryological work in taxonomy.

Literature Cited

Anderson, L. E., 1936. Mitochondria in the life cycles of certain higher plants. Amer. J. Bot. *23*, 490-500.

Banerji, I. and Gangulee, H. C., 1937. Spermatogenesis in *Eichhornia crassipes* Solms. Jour. Ind. Bot. Soc. *16*: 289-296.

Billings, F. H., 1934. Male gametophyte of *Atriplex hymenelytra*. Bot. Gaz. *95*, 477-484.

Blackman, V. H. and E. J. Welsford, 1913. Fertilization in *Lilium*. Ann. Bot. *27*, 111-114.

Bobilioff-preisser, W., 1917. Beobachtungen an isolierten Palisaden—und Schwammparenchymzellen. Beih. bot. Centralbl. *33 A*, 248-274.

Campbell, D. H., 1898. Development of flower and embryo in *Lilaea subulata*. Ann. Bot. *12*, 1-28.

Capoor, S. P., 1937. The life history of *Holoptelea integrifolia* Planch. Beih. bot. Centralbl. 57A: 233-249.

Chamberlain, C. J., 1897. The pollen grain. In: Coulter, J. M., C. J. Chamberlain and J. H. Schaffner, Contributions to the life history of *Lilium philadelphicum*. Bot. Gaz. *23*, 412-452.

Cheesman, E. E., 1927. Fertilization and embryogeny in *Theobroma cacao*. L. Ann. Bot. *41*, 107-126.

Cooper, D. C., 1935. Microsporogenesis and the development of the male gametes in *Portulaca oleracea*. Amer. J. Bot. *22*, 453-459.

————1936. Development of the male gametes of *Lilium*. Bot. Gaz. *98*, 169-177.

Cooper, G. O., 1935. Cytological studies in the Chenopodiaceae. I. Microsporogenesis and pollen development. Bot. Gaz. *97*, 169-178.

————1936. Cytological investigations of *Erechtites hieracifolia*. Bot. Gaz. *98*, 348-355.

Coulter, J. M., and C. J. Chamberlain, 1903. Morphology of Angiosperms. New York.

Dahlgren, K. V. O., 1916. Zytologische und embryologische Studien über die Reihen Primulales und Plumbaginales. Kungl. Svenska Vetensk. Akad. Handl. *56*.

————1927. Die Befruchtungserscheinungen der Angiospermen. Hereditas *10*, 169-229.

DRAHOWZAL, G., 1936. Beiträge zur Morphologie und Entwicklungsgeschichte der Pollenkörner. Österr. bot. Z. *85*, 241-269.

DUGGAR, B. M., 1900. Studies in the development of the pollen grain in *Symplocarpus foetidus* and *Peltandra undulata*. Bot. Gaz. *29*, 81-98.

EICHHORN, A., 1933. Sur la prétendue existence de centrosomes et d'asters chez les végétaux supérieurs. C.r. Acad. Sci. *196*, 1239-1241.

ELFVING, F., 1879. Studien über die Pollenkörner der Angiospermen. Jenaische Z. *13*, 1-28.

FEDORTSCHUK, W., 1931. Embryologische Untersuchung von *Cuscuta monogyna* VAHL und *Cuscuta epithymum* L. Planta *14*, 94-111.

FENG, Y.-A., 1932. Sur la présence de centrosomes et d'asters chez une Angiosperme, *Lonicera alpigena*. C.r. Acad. Sci. *194*, 2317-2319.

————1934. Recherches cytologiques sur la caryocinèse, la spermatagenèse et la fécondation chez les Caprifoliacées. Le Botaniste *26*, 1-88.

FINN, W. W., 1925. Male cells in Angiosperms. Bot. Gaz. *80*, 1-25.

————1926. Spermazellen bei *Vincetoxicum*. Ber. deutsch. bot. Ges. *44*, 133-137.

————1928a. Spermazellen bei *Vinca minor* und *V. herbacea*. Ber. deutsch. bot. Ges. *46*, 235-246.

————1928b. Über den Pollenschlauch bei *Fagus silvatica*. Nawaschin Festschrift. 63-66.

————1935a. Einige Bemerkungen über den männlichen Gametophyten der, Angiospermen. Ber. deutsch. bot. Ges. *53*, 679-686.

————1935b. Streitfragen in der Entwicklung des männlichen Gametophyten der Angiospermen. Bull. Sci. Univ. Etat Kiev *1*, 25-47.

————and T. RUDENKO, 1930. Spermatogenesis und Befruchtung bei einigen Orobanchaceae. Bull. Jard. Bot. Kieff. *11*, 69-82.

————and L. D. SAFIJOWSKA, 1933. Zur Embryologie und Karyologie der Gattung *Cuscuta*. Bull. Jard. Bot. Kieff. *16*, 51-66.

FRIEMANN, W., 1910. Über die Entwicklung der generativen Zelle im Pollenkorn der monocotylen Pflanzen. Diss. Bonn.

FRISENDAHL, A., 1912. Cytologische und entwicklungsgeschichtliche Studien über *Myricaria germanica*. Kungl. Svenska Vetensk. Handl. *48*.

——————1927. Über die Entwicklung chasmo-und kleistogamer Blüten bei der Gattung *Elatine*. Medd. Göteborgs bot Trädgard *3*, 99-142.

FUCHS, A., 1936. Untersuchungen über den männlichen Gametophyten von *Elaeagnus angustifolia*. Österr. bot. Z. *85*, 1-16.

GAGER, C. S., 1902. The development of the pollinium and sperm-cells in *Asclepias cornuti* DECAINE. Ann. Bot. *16*, 123-148.

GEITER, L., 1935. Beobachtungen über die erste Teilung im Pollenkorn der Angiospermen. Planta *24*, 361-386.

GEITLER, L., 1937. Zur Morphologie der Pollenkörner von *Clarkia elegans*. Planta *27*: 426-431.

GERASSIMOWA, H., 1933. Fertilization in *Crepis capillaris*. La Cellule *42*, 103-148.

GOEBEL, K., 1933. Organographie der Pflanzen. III. Samenpflanzen. 3rd edition. Jena.

GORCZYNSKI, T., 1929. Badania histo-cytologiczne nad kwiatami kleistogamicznemi u *Lamium amplexicaule, Oxalis acetosella* i *Viola odorata* (Recherches histo-cytologiques sur les fleurs cléistogames chez *Lamium amplexicaule, Oxalis acetosella* et *Viola odorata*). Acta Soc. Bot. Poloniae *6*, 248-295.

GUIGNARD, L., 1891. Nouvelles études sur la fécondation. Ann. Sci. Nat., Bot., Sér. *7*, *14*, 163-296.

GUILLIERMOND, A., 1920. Sur l'évolution du chondriome pendant la formation des grains de pollen de *Lilium candidum*. C.r. Acad. Sci. *170*, 1003-1006.

——————1924. Recherches sur l'évolution du chondriome pendant le développement des grains de pollen et du sac embryonnaire chez les Liliacées et sur la signification des formation ergastoplasmiques. Ann. Sci. Nat., Bot., Sér. 10, *6*, 1-52.

GURGENOWA, M., 1928. Fertilization in *Phelipaea ramosa*. Nawaschin Festschrift 157-168.

HÅKANSSON, A., 1924. Beiträge zur Zytologie eines *Epilobium*bastards. Bot. Notiser, 169-278.

HEITZ, E., and F. RESENDE, 1936. Zur Methodik der Pollenkorn—und Pollenschlauchuntersuchung. Bol. Soc. Broteriana *11*, 5-15.

HERRIG, F., 1919. Über Spermazellen im Pollenschlauch der Angiospermen. Ber. deutsch. bot. Ges. *37*, 450-453.

——————1922. Über Fragmentation und Teilung der Pollenschlauchkerne von *Lilium candidum*. Beitr. Allg. Bot. *2*, 403-412.

HEUSSER, K., 1915. Die Entwicklung der generativen Organe von *Himantoglossum hircinum*. Beih bot. Centralbl. *32 A*, 218-277.

HOARE, G. V., 1934. Gametogenesis and fertilization in *Scilla nonscripta*. La Cellule *42*, 269-291.

ISHIKAWA, M., 1918. Studies on the embryo sac and fertilization in *Oenothera*. Ann. Bot. *32*, 279-317.

JOHRI, B. M., and S. NAND, 1935. The development of the male and female gametophytes in *Cuscuta reflexa*. Proc. Indian Acad. Sci., B, *1*, 283-289.

JOHRI, B. M., 1936. The life-history of *Butomopsis lanceolata* Kunth. Proc. Ind. Acad. Sci. B, *4:* 139-162.

JOSHI, A. C. and J. VENKATESWARALU, 1936. Embryological studies in the Lythraceae. III. Proc. Indian Acad. Sci., B, *3*, 377-400.

JOSHI, P. C., 1936. Contribution to the life-history of *Stellaria media* L. Proc. Indian Acad. Sci., B, *3*, 8-22.

JUNELL, S., 1931. Die Entwicklungsgeschichte von *Circaeaster agrestis*. Svensk. bot. Tidskr. *25*, 238-270.

KIYOHARA, K., 1935. Zur SCHIMPER-MEYERSCHEN Theorie der Vermehrung der Chloroplasten. J. Fac. Sci. Imp. Univ. Tokyo, Sect. III. Bot. *4*, 399-465.

KOERNICKE, M., 1906. Zentrosomen bei Angiospermen? Flora *96*, 501-522.

KRUPKO, S., 1926. Les plastides et le chondriome pendant la gonogenèse dans le *Gagea lutea*. Acta Soc. Bot. Poloniae *4*, 77-86.

LAGERBERG, F., 1909. Studien über die Entwicklungsgeschichte und systematische Stellung von *Adoxa moschatellina*. Kungl. Svenska Vetensk. Handl. *44*.

LOCKE, J. F., 1936. Microsporogensis and cytokinesis in *Asimina triloba*. Bot. Gaz. *98*, 159-168.

MADGE, M., 1929. Spermatogenesis and fertilization in the cleistogamous flower of *Viola odorata var. praecox*. Ann. Bot. *43*, 545-577.

——————1936. Division of the generative cell in *Hedychium Gardnerianum*. La Cellule *45*, 171-176.

MAHESHWARI, P., 1931. Contribution to the morphology of *Albizzia Lebbek*. J. Indian Bot. Soc. *10*, 241-264.

————and H. D. WULFF, 1937. Recent advances in microtechnic. I. Methods of studying the development of the male gametophyte of Angiosperms. Stain Tech. *12*: 61–70.

MANSHARD, E. 1936. Embryologische Untersuchungen an *Styrax obassia* SIEB. et ZUCC. Planta *25*, 364-383.

MERRELL, W. D., 1900. A contribution to the life history of *Silphium*. Bot. Gaz. *29*, 99-133.

MODILEWSKI, J., 1918. Cytological and embryological studies on *Neottia nidus avis* (L.) RICH. Mém. Soc. Nat. Kiew *26*, 1-55.

MOHRBUTTER, C., 1936. Embryologische Studien an Loganiaceen. Planta *26*, 64-80.

MÜLLER, H., 1936. Zytologische Untersuchungen über die Haploidgeneration der Apocynaceen. Diss. Berlin.

MURBECK, S., 1902. Über die Embryologie von *Ruppia rostellata*. Kungl. Svenska Vetensk. Akad. Handl. *36*.

NAITHANI, S. P. (1937). Chromosome studies in Hyacinthus orientalis L. III. Reversal of sexual state in the anthers. Ann. Bot. (N. S.), *1:* 369-378.

NAWASCHIN, S. G., 1897. Über die Befruchtung bei *Junglans regia* und *J. nigra*. Trav. Soc. Imp. Nat. St. Pétersbourg *28*.

————1909. Über dasselbständige Bewegungsvermögen des Spermakerns bei einigen Angiospermen. Österr. bot. Z. *59*, 457-467.

————1910. Näheres über die Bildung der Spermakerne bei *Lilium martagon*. Ann. Jard. Bot. Buitenzorg. 3. Suppl., 871-904.

————1927. Essai de représentation structurelle des propriétés des noyaux sexuels. Melanges botaniques offerts à Mr. I. BORODINE, Leningrad, 94-114.

————and W. W. FINN, 1912. Zur Entwicklungsgeschichte der Chalazogamen. Mém. Soc. Nat. Kieff *22*.

NĚMEC, B., 1898. O pylu petaloinich tyčinek hyacintu (*Hyacinthus orientalis* L.) Über den Pollen der petaloiden Antheren von *Hyacinthus orientalis* L. Rozpravy Ceske Akad. Prag, II, *7*.

NEWMAN, I.V., 1934. Studies in the Australian Acacias. III. Supplementary observations on the habit, carpel, spore production and chromosomes of *Acacia Baileyana*. F. v. M. Proc. Linnean Soc. New South Wales *59*, 237-251.

OEHLER, E., 1927. Entwicklungsgeschichtlich-cytologische Untersuchengen an einigen saprophytischen Gentianaceen. Planta *3*, 641-733.

OLIVER, F. W., 1891. On *Sarcodes sanguinea*. Ann. Bot. *2*, 75-115.

O'MARA, J., 1933. Division of the generative nucleus in the pollen tube of Lilium. Bot. Gaz. *94*, 567-578.

OSTERWALDER, A., 1898. Beiträge zur Embryologie von *Aconitum napellus*. Flora *85*, 254-292.

OVERTON, E., 1891. Beitrag zur Kenntnis der Entwicklung und Vereinigung der Geschlechtsprodukte bei *Lilium martagon*. Festschrift für NAEGELI und KOLLIKER, Zürich.

PERSIDSKY, D., 1926. Zur Embryologie der *Orobanche cumana* WALLR. und der *O. ramosa* L. Bull. Jard. Bot. Kieff *4*, 6-10.

PETER, K., 1930. Die Beziehungen zwischen Zellteilung und Zelltätigkeit, Darstellung und Versuch einer kausalen Betrachtung. Sammelreferat. Protoplasma *10*, 613-625.

PIECH, K., 1924a. Zur Entwicklung der Pollenkörner bei *Scirpus lacustris*. Bull. Acad. Polon. Sci. et Lettr., 113-123.

————1924b. Über die Teilung des primären Pollenkerns und die Entstehung der Spermazellen bei *Scirpus paluster*. Bull. Acad. Poln. Sci. et Lettr., 605-621.

————1928. Zytologische Studien an der Gattung *Scirpus*. Bull. Acad. Polon. Sci. et Lettr., Sér. B. 1-43.

PODDUBNAJA-ARNOLDI, W. A., 1927. Spermatogenesis bei einigen Compositen. Planta *4*, 284-298.

————1933. Künstliche Kultur und zytologische Untersuchung des Pollenschlauches von *Senecio platanifolius*. Planta *19*, 299-304.

————1934. Spermazellen in der Familie der Dipsacaceae. Planta *21*, 381-386.

————1936. Beobachtungen über die Keimung des Pollens einiger Pflanzen auf künstlichem Nährboden. Planta *25*, 502-529.

PURI, V., and B. SINGH, 1935. Studies in the family Amaranthaceae. I. The life-history of *Digera arvensis* FORSK. Proc. Indian Acad. Sci., B, *1*, 893-908.

RUDENKO, T., 1929. Bildung der Spermazellen bei *Scrophularia Nodosa* L. und *S. alata* GILIB. bei der Teilung der generativen Zelle im Pollenschlauch. Bull. Jard. Bot. Kieff *9*, 18-30.

————————1930. Male cells of Scrophulariaceae. Spermatogenesis and fertilization by *Lathraea squamaria*. Bull. Jard. Bot. Kieff *11*, 41-55.

————————1933. Male cells of Scrophulariaceae. Bull. Jard. Bot. Kieff *16*, 1-16.

RUHLAND, W. and K. WETZEL, 1924. Der Nachweis von Chloroplasten in den generativen Zellen von Pollenschläuchen. Ber. deutsch. bot. Ges. *42*, 3-14.

SAFIJOWSKA, L. D., 1934. Zur Embryologie der *Adenophora liliifolia* LED. J. Inst. Bot. Acad. Sci. Ukraine *11*, 85-98.

————————1935. Spermatogenesis bei Campanulaceen. Bull. Sci. Etat Kiev, 265-278.

SAMUELSSON, G., 1913. Studien über die Entwicklungsgeschichte einiger Bicornes-Typen. Svensk bot. Tidskr. *7*, 97-188.

SAWYER, M. L., 1917. Pollen tubes and spermatogenesis in *Iris*. Bot. Gaz. *64*, 159-164.

SAX, K., 1935. The effect of temperature on nuclear differentiation in microspore development. J. Arnold Arbor. *16*, 301-310.

————————and H. W. EDMONDS, 1933. Development of the male gametophyte in *Tradescantia*. Bot. Gaz. *95*, 156-163.

————————and L. HUSTED, 1936. Polarity and differentiation in microspore development. Amer. J. Bot. *23*, 606-609.

SCHAFFNER, J. H., 1896. The embryo sac of *Alisma plantago*. Bot. Gaz. *21*, 123-132.

————————1897. The life-history of *Sagittaria variabilis*. Bot. Gaz. *23*, 252-273.

SCHNARF, K., 1929. Embryologie der Angiospermen. Berlin.

SCHNIEWIND-THIES, J., 1901. Die Reduktion der Chromosomenzahl und die folgenden Kernteilungen in den Embryosack-mutterzellen der Angiospermen. Jena.

SCHURHOFF, P. N., 1921. Über die Teilung des generativen Kernes vor der Keimung des Pollenkorns. Arch. Zellforsch. *15*, 145-159.

————————1922. Die Befruchtung von *Viscum album*. Ber. deutsch. bot. Ges. *40*, 314-316.

————————1926. Die Zytologie der Blütenpflanzen. Stuttgart.

SHARP, L.W., 1934. Introduction to cytology. 3rd edition. New York and London.

SHATTUCK, C. H., 1905. A morphological study of *Ulmus americanus*. Bot. Gaz. *40*, 209-223.

SHIBATA, K. 1902. Die Doppelbefruchtung bei *Monotropa uniflora*. Flora *90*, 61-66.

SMITH, C. M., 1929. Development of *Dionaea muscipula*. Bot. Gaz. *87*, 508-530.

SMITH, W. R., 1898. A contribution to the life-history of the Pontederiaceae. Bot. Gaz. *25*, 324-337.

STOW, I., 1930. Experimental studies on the formation of the embryosac-like gian pollen grains in the anther of *Hyacinthus orientalis*. Cytologia *1*, 417-439.

——————1933. On the female tendencies of the embryosac-like giant pollen grain of *Hyacinthus orientalis*. Cytologia *5*, 88-108.

STRASBURGER, E., 1884. Neuere Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen als Grundlage für eine Theorie der Zeugung. Jena.

——————1888. Über Kern-und Zellteilung im Pflanzenreiche, nebst Anhang über Befruchtung. Hist. Beitr. *1*, Jena.

——————1900. Einige Bemerkungen zur Frage nach der "Doppelten Befruchtung" bei den Angiospermen. Bot. Z. *58*, 293-316.

——————1908. Chromosomenzahlen, Plasmastrukturen, Vererbungsträger und Reduktionsteilung. Jahrb. Bot. *45*, 477-570.

SUITA, N., 1937a. On the mature pollen grains in Angiosperms. Bot. Mag. Tokyo *51*: 524–529.

——————1937b. Studies on the male gametophyte in Angiosperms, II. Differentiation and behaviour of the vegetative and generative elements in the pollen grains of *Crinum*. Cytologia. Fujii Jubl. Vol. 920-933.

TRANKOWSKY, D. A., 1931. Zytologische Bebachtungen über die Entwicklung der Pollenchläuche einiger Angiospermen. Planta *12*, 1-18.

TSCHERNOJAROW, M., 1926. Befruchtungserscheinungen bei *Myosurus minimus*. Österr. bot. Z. *75*, 197-206.

UPCOTT, M., 1936. The mechanics of mitosis in the pollen-tube of *Tulipa*. Proc. R. Soc. London, B, *121*, 207-220.

VALKANOV, A., 1933. Über die kinetische Energie einiger Zellbestandteile. Protoplasma *20*, 20-30.

WEFELSCHEID, G., 1911. Über die Entwicklung der generativen Zelle im Pollenkorn der dikotylen Angiospermen. Diss. Bonn.

WEINZIEHER, S., 1914. Beiträge zur Entwicklungsgeschichte von *Xyris indica*. L. Flora N. F. *6*, 393-432.

WELSFORD, E. J., 1914. The genesis of the male nuclei in *Lilium*. Ann. Bot. *28*, 265-270.

WEST, G., 1930. Cleistogamy in *Viola Riviniana*, with especial reference to the cytological aspects. Ann. Bot. *44*, 87-109.

WÓYCICKI, Z., 1911. Die Endphasen der Pollenentwicklung bei *Yucca recurva*. C.r. Soc. Sci. Varsovie *3*, 17-23.

——————1926. Grains de pollen, tubes polliniques et spermatogenèse chez *Haemanthus Katharinae* BAK. Bull. Acad. Polon. Sci. et Lettr., B, 177-188, 535-557.

WULFF, H. D., 1933. Beiträge zur Kenntnis des männlichen Gametophyten der Angiospermen. Planta *21*, 12-50.

——————1934*a*. Untersuchungen an Pollenkörnern und Pollenschläuchen von *Impatiens parviflora*. Ber. deutsch. bot. Ges. *52*, 43-47.

——————1934*b*. Ein Beitrag zur Bedeutung der Trabantenchromosomen. Ber. deutsch. bot. Ges. *52*, 597-606.

——————1935. Ein Vergleich zwischen Kultur-und Griffelpräparaten von Pollenschläuchen von *Narthecium ossifragum*. Beih. bot. Centralbl. *54 A*, 83-98.

——————and M. LINDSCHAU, 1936. Über die Kern-Plasma-Relation in den generativen Zellen der Angiospermen-Pollenkörner. Planta *25*, 151-154.

——————and T. S. RAGHAVAN, 1937. Beobachtungen an Pollenschlauchkulturen von des Hydrophyllacee *Nemophila insignis*. Planta *27* : 466-473.

WUNDERLICH, R., 1936. Vergleichende Untersuchungen von Pollenkörnern einiger Liliaceen und Amaryllidaceen. Österr. bot. Z. *85*, 30-55.

WUNDERLICH, R., 1937. Zur Vergleichenden Embryologie der Liliaceae-Scilloideae. Flora N. F., *32*: 48-90.

WYLIE, R. B., 1904. The morphology of *Elodea canadensis*. Bot. Gaz. *37*, 1-22.

——————1923. Sperms of *Vallisneria spiralis*. Bot. Gaz. *75*, 191-202.

INVESTIGATIONS ON ORANGE ROT IN STORAGE

I. Orange rot due to two strains of *Fusarium moniliforme* Sheldon

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Calcutta, in winter, gets an abundant supply of oranges (*Citrus chrysocarpa* Lush) (7) commonly known as *Kamalá* in Bengal from Darjeeling and neighbouring areas, Assam particularly Sylhet and Khasi Hills and Nagpur in the Central Provinces. The fruits are usually packed in baskets or in wooden boxes and transported by train or steamer to Calcutta. A large proportion of the fruits are found to be damaged by different types of rots due to moulds during transit and subsequent storage in Calcutta and consequently the dealers suffer a certain amount of loss every year.

During an investigation on such rots the writer came across a particular type caused by two strains of *Fusarium moniliforme* Sheldon. Strain A1 was found on Darjeeling and strain A2 on Assam oranges. In this paper an attempt has been made to give an account of the symptoms on the fruits brought about by these fungi, their morphological characters and also their position in relation to the host tissue. Further work is in progress to find out the exact conditions which favour this rot and also some suitable means of its control. Results of these investigations will be published in a subsequent paper. Both the fungi were identified to be *Fusarium moniliforme* Sheldon, which were verified from Centraalbureau Voor Schimmelculture, Baarn, Holland.

Examination of Darjeeling and Assam oranges, opened immediately after arrival, showed some of them developing white patches surrounded by water soaked areas near the stem end or on other parts. Small semipliable and light brown areas were observed in certain cases. In more advanced stages of rotting the white portion

extended further and the water soaked region also occupied a bigger area and turned dark brown (Pl. 1 A & B). The whole fruit later on developed a soft rot and turned into a pulpy mass (Pl. 1 C). Several consignments were examined during the winter from November to January and the number of fruits developing such rots varied from 10 to 20% or sometimes more in Darjeeling and Assam varieties whereas the Nagpur oranges were almost free from it.

Fruits with moulds or water soaked area alone were collected from different consignments and brought to the laboratory in sterile containers. On being kept for further observation, the highly infected ones specially those with well developed white patch were found to disintegrate in 2 to 3 days' time and that was always associated with exudation of a liquid with bad odour. The less infected fruits with brown spots alone, developed white areas in a day or two. Rotting proceeded in the same way as was observed in the highly infected ones. Microscopic examination of the rotted portion from the interior showed fungal hyphae permeating the host cells and the white patches on the surface of the rind containing innumerable conidia.

Isolation and Culture

Isolations from infected fruits of both localities were made. Surface sterilisation was effected by washing with a saturated solution of borax, steeping in ·01% mercuric chloride solution for 5 minutes and finally washing in sterile distilled water (1). A slit was made a little away from the periphery with a flamed scalpel and a little bit of the host tissue quickly transferred to petridishes containing Glucose Asparagin medium. Conidia also were taken from the surface of the rind and cultured in the same way as before.

When pure cultures were obtained, several sub-cultures were made on different stock media. Growing mycelium and conidia formations were examined *in situ* on Coon's medium which seemed most suitable for its relative transparency.

To study the external morphology, dilute cotton blue was used which stained the cytoplasm leaving the walls unstained.

Morphology of the Fungi

Both the fungi have similar morphological characters (9) with slight variations in cultural features. The mycelium is widely spreading, floccose and white in colour in the primary stage, gradually changing into salmon pink. The hyphae are much branched, septate and narrow with a thickness of $6-8\mu$ (Fig. 1/-). The growth of the mycelium in strain A_1 is more vigorous than in A_2. Two types of conidia are found. The macro conidia are 3–4 septate (Fig. 3/-), falcate with two ends tapering, occurring in a bunch on a short conidiophore. The average size of the macro conidia is

25μ—80μ in length. The micro conidia is more abundant and is formed on branched conidiophores (Fig. 1). They are ovoid, moniliform and 6–10μ in length. Though the proportion of micro-conidia is much higher than that of macro-conidia in both the strains, in strain A_1 however percentage of macro-conidia is much higher than that in A_2.
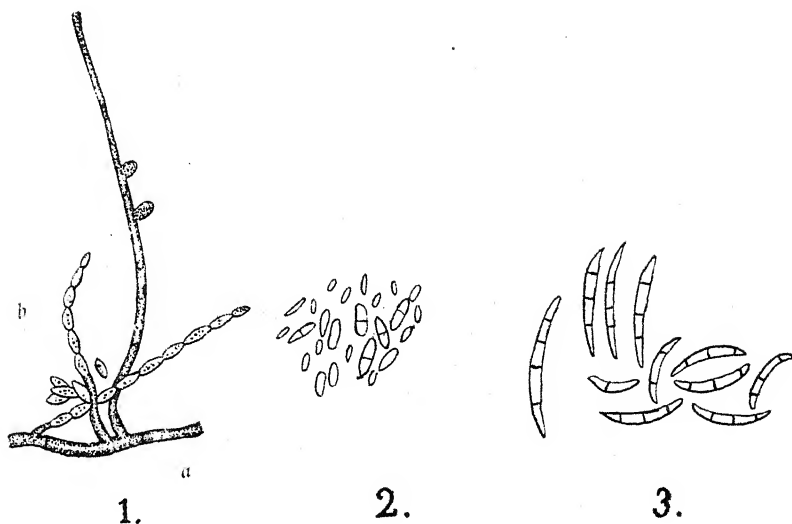
Fig. 1. *a*. Hyphae, *b*. microconidiophore with microconidia. $\times 625$.
Fig. 2. Microconidia. $\times 625$.
Fig. 3. Macroconidia. $\times 625$.

Inoculation

In order to establish their pathogenicity and also to test whether these strains of Fusarium isolated from rotten oranges were responsible for bringing about complete rot, a series of inoculations were made with pure cultures of the fungi on healthy oranges of different ages. Before such inoculations, precautions were taken to keep the surface of the fruit in aseptic condition by treating with $0\cdot01\%$ mercuric chloride and washing several times with sterile distilled water. The fruits were then kept in large petridishes lined with wet blotting paper previously sterilised.

Two series of inoculations were made with each fungus. In the "injured series," wounds of different magnitude were cut on fresh ripe oranges and inoculated with hyphae and conidia. In certain cases conidia in suspension were sprayed on the surface of the fruits particularly on the stem end with wounds. Both the

methods were successful in bringing about infection. In everyone of these inoculations where the fungi were introduced through wounds, the rot was successfully induced and it increased with the size and depth of the injury. The pathogens on re-isolations were found to be the same with which the fruits were inoculated.

In the "uninjured series", hyphae and conidia were put on the uninjured rind but in no case the fungi could penetrate the outer skin and produce any rot. In certain cases a small amount of culture medium was put along with the fungus but no infection followed. Mycelium grew so long as the medium lasted but later on dried up.

Inoculation experiments were carried out with green oranges as well. A marked difference in susceptibility was observed in the primary stages of infection. They were attacked but the progress was very slow to start with. It took comparatively longer period for the fungi to appear with their fructifications on the surface of the fruits. The ripe fruit on the other hand were quickly attacked and the progress of rotting was very fast. Perhaps this variation in susceptibility might be due to the difference in acid and sugar contents of the fruits of different ages. The growth of these fungi is being studied in synthetic media with different concentrations of acid and various combinations of sugars commonly present in oranges, the result of which will be published in another paper.

In order to test their power of infection on oranges of different localities, cross inoculations were done, results of which are given in a tabular form below:

Fungus	Oranges of different localities	No. of oranges inoculated	No. of oranges infected	REMARKS
A_1	Darjeeling ..	10	10	Vigorous infection.
	Assam ..	10	10	Very vigorous infection.
	Nagpur ..	10	2	Very slight infection and in many cases nil.
A_2	Darjeeling ..	10	3	Very mild infection.
	Assam ..	10	10	Vigorous infection.
	Nagpur ..	10	Nil.	Nil.

The above table proves that strain A_1 is more virulent than A_2 as regards their power of infection is concerned. The former

can infect Assam and Darjeeling varieties and in certain cases Nagpur as well, but the progress of decay in the last case is very slow and often checked after a short period. Strain A_2 on the other hand causes decay of Assam and Darjeeling oranges but does not infect Nagpur varieties.

Course of fungal hyphae in relation to host tissue

Inoculation experiments proved beyond doubt that the fungi causing such soft rots were wound parasites. After its entrance into the host tissue the mycelium travelled across the whole thickness of the rind and ramified abundantly on the inner side adjoining the juice vesicles (Fig. 4). Hyphae attacked the cells

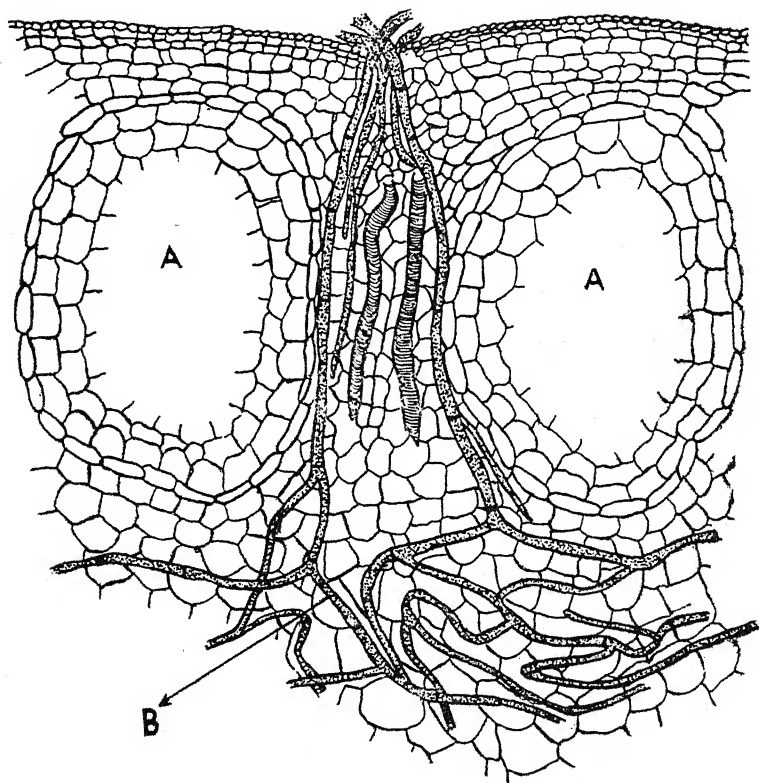Fig. 4. Section of the decaying orange rind. A. oil vesicle; B. filaments of the fungus ramifying within the tissues. ×173.

surrounding the oil glands and they were found to be both intra and intercellular (5). No special absorbing organs were noticed.

Decomposition of the host tissue started from within and a clear case of killing in advance of penetration was observed in every case. This was perhaps the reason why the softening of the tissue was much ahead of the advancement of the hyphae. Such killing and decomposition were due to pectinase enzyme secreted by the invading hyphae. The enzyme was extracted from fresh cultures of the fungi and their action studied by injecting into fresh oranges. Disintegration of the injected tissue followed quickly in a few hours but the controls injected with distilled water alone showed no signs of decay. In the final stage the pathogens produced conidia on the surface of the fruits.

Discussion and Conclusion

Stevenson (6) mentioned a pink stylar end rot associated with *Fusarium* in addition to the brown or black one due to *Alternaria*. While making an investigation of decay in *Citrus* fruits from California in 1924 Fawcett (3) isolated *Fusarium* occasionally, especially from decayed lemon fruits that had been picked for a long time.

Strains of *Fusarium* identified as *F. oxysporum, F. fructigenum, F. lateratium* were isolated from a pliable leathery type of stem end rot of *Citrus* fruits in California.

Fusarium lateratium Nees was found causing a minor decay on oranges in Italy and Sicily.

Fusarium rot has been reported in California, Texas, Puerto Rico, Lesser Antillis, Italy, Cyprus, Palestine, Tunisia, Algeria, South Africa, Southern Rhodesia, Kenya Colony, New South Wales, Western Australia, Japan, China and Brazil (4).

In India little had been done on *Citrus* fruit rot until recent years. Chaudhuri (2) in Northern India found *Citrus* fruit rot due to *Colletotrichum gloeosporioides*. In Central (8) and South India *Citrus* fruit rots were found to be due to *Phytophthora palmivora* which causes a brown rot.

The present investigation shows that the Darjeeling and Assam oranges are susceptible to *Fusarium* rot. The two strains showed marked difference in their parasitic activities. By cross inoculation it has been found out that strain A_1 is more virulent than A_2 hence they have been classified into two different physiologic forms.

The infecting fungi, though they may differ in certain characters, are both wound parasites. None of them could infect a healthy fruit unless there was a wound present either at the stem end or on the rind. Inoculations by spraying or putting the pathogens along with a certain amount of culture medium failed to induce infection on an uninjured rind.

As regards the wastage caused by these fungi, the Assam oranges showed the highest percentage. Strain A₂ though comparatively less virulent, was potent enough to cause serious damage to Assam varieties. Strain A₁ infected both Darjeeling and Assam oranges but it was more virulent in the latter. The Nagpur oranges practically suffered no loss due to strain A₂. Infection was induced with A₁ but the percentage was comparatively smaller and the progress of decay very slow.

Summary

1. Oranges sold in Calcutta market in winter are damaged by different types of rots due to fungi. Two strains of *Fusarium moniliforme* Sheldon are found to be responsible for causing a soft rot on a large number of these fruits.

2. Oranges from Darjeeling and Assam suffer more from this rot, whereas the Nagpur variety is almost free from it. Strain A₁ was isolated from Darjeeling and A₂ from Assam oranges.

3. The disease appears as a small semipliable water soaked area on the rind with light brown colour and later on with white patches in the centre. In the final stage the whole surface is covered with white incrustations and the fruit turns into a pulpy mass.

4. Both strains have similar morphological features with slight variations in cultural characters. The growth of the mycelium in strain A₁ is more vigorous than in A₂. The average size of the macro and micro conidia varies in length from 25–80μ and 6–10μ respectively. The proportion of micro conidia is much higher than that of macro conidia in both the strains but in strain A₁ the percentage of macro conidia is much higher than that in A₂.

5. That the fungi are strictly wound parasites were proved by inoculation experiments.

6. Cross inoculations showed strain A₁ to be more virulent. It could infect Assam or Darjeeling oranges readily and in certain cases Nagpur variety as well. Strain A₂ could infect only Assam oranges vigorously. It was less virulent on Darjeeling variety, and Nagpur oranges were practically immune to it.

7. It is for their difference in pathogenicity mainly that the fungi have been classified into two physiologic forms.

8. The fungal hyphae entering into the host tissue ramify in the inner side of the rind adjoining the juice vesicles. Rotting starts from within and conidia appear finally as white incrustations on the surface of the fruit.

Literature Cited

1. BAKER, R. E. D. and WARDLAW, C. W. On the types of infection encountered in the storage of certain fruits. *Annals of Botany.* New series. Vol. 1, No. 1, 1937.

2. CHAUDHURI, H., and SINGH, G. The withertip disease of Citrus plants. *Journal and Proc. Asiatic Soc., Bengal.* New series. 26 : 523–532. 3 pls. 1933.

3. FAWCETT, H. S. The decay of Citrus fruits on arrival and in storage at eastern markets. *California Citrograph.* 10 : 79, 98, 99, 103, 5 figs. 1925.

4. ————Citrus diseases and their control, 1936.

5. SMITH, R. E. The 'soft spot' of oranges. *Bot. Gaz.*, 24 : 103–104. 1897.

6. STEVENSON, JOHN A. A check list of Puerto Rican Fungi and a host index. *Jour. Dept. Agr., Puerto Rico,* 2: 125–264. 1918.

7. TANAKA, TYOZABURO. Further revision of Rutaceae—Aurantioidae of India and Ceylon. *The Journal of the Indian Botanical Society,* Vol. XVI, No. 4. 1937.

8. UPPAL, B. N. Summary of the work done under the plant pathologist. *Dept. Agr. Bombay Presidency Anu. Report.* 1930-33.

9. WOLLENWEBER, DR. H. W., UND REINKING, DR. O. A. "Die Fusarien". 1935.
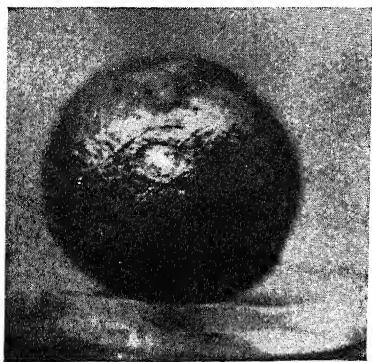
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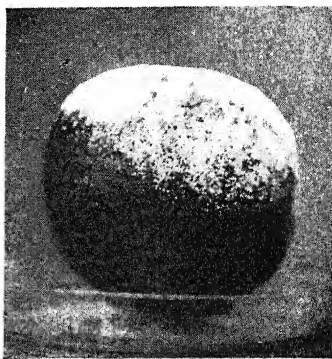
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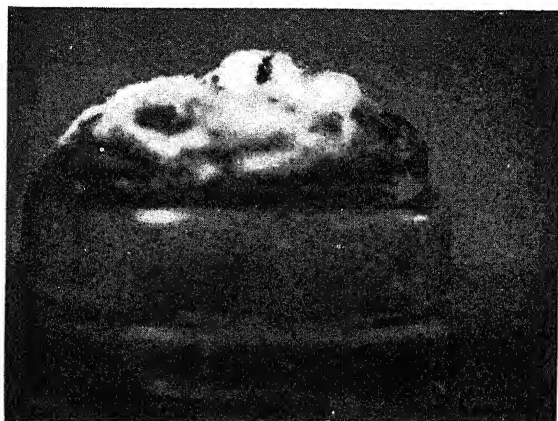
C

ISOELECTRIC POINT OF THE PROTEINS OF THE TISSUES OF THE COTTON PLANT IN RELATION TO THE pH OF THE CELL SAP

BY

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The cotton is an important economic crop in the Bombay Presidency where it is grown on a large area. It has, therefore, been a subject of intensive study with the Agricultural Department from different points of view. These studies have already yielded results of practical value. The object of this investigation is not economic but academic. It is also not attempted, at present, to correlate the physiological property of the plant with some external feature of its growth as done by Hawkins and his colleagues (1934) who have shown that osmotic pressures and percentage sheddings of bolls are correlated. It is intended to study the physico-chemical nature of the tissues of the cotton plant so as to obtain an insight into the modes of functioning.

A plant tissue is an aggregate of biological colloids of protein nature, and the intake of ions and water depends on the physical properties of the colloids. It is, therefore, of paramount importance that before we attempt to understand the entry of water and salts in the plant tissues it is necessary to know something of the substances on whose physical properties the entry of water and salts very much depends. It is this knowledge that will give us true insight into the water and the salt requirements of the plants and the best conditions necessary in which they become available to the plants. For the important crop plant like the cotton the need for such a biochemical study need not be too strongly emphasised.

It was, therefore, considered of importance to study some important physico-chemical properties of the tissues of the roots, stem, leaves and bolls of the crop plant like the cotton plant, namely, the hydrogen ion concentration and the isoelectric point of the proteins. No such study has been attempted before in the case of the cotton plant growing in India.

The cotton plant is not cultivated in the Konkan and it was therefore not advisable to work on these aspects of physiology of the cotton plant grown in Bombay. In order to get reliable data it was necessary to study the cotton plant in the normal place of cultivation.

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It was, therefore, necessary to carry on this investigation with the plants grown at some place other than Bombay, or its suburbs. After a careful consideration of the several districts in which the cotton is cultivated Surat district was selected for the purpose, it being a place nearest Bombay.   The existence of the Government Agricultural Farm at Athwa Lines was another consideration for carrying on the investigations at Surat.   The Deputy Director of Agriculture, Surat, kindly provided facilities for the work, and a field-area containing about 400 plants of the commonly grown variety 1027 A.L.F. of *G. herbaceum* cotton was placed at our disposal.

Methods

As the cell sap of the tissues of the cotton plant is below pH 7, the quinhydrone electrode (Leeds and Northrup type 7701) was used in preference to hydrogen electrode for determining the pH of the sap.   The voltage of solutions was measured by Poggenderf's compensation method with a portable potentiometer (Cambridge Instrument Co.).   The apparatus was tested every week before taking a reading with two standard buffer solutions (1) N/20 Sodium Borate and (2) universal buffer mixture powder of B.D.H.

Plants were dug out every week at about 6 A.M. (before sunrise).   The roots were thoroughly washed first with tap water and then with distilled water.   The sap was extracted separately from the different parts of the plant by the method of Ingalls and Shive (1931).   15 ccs. of sap was obtained at each extraction and diluted to 30 ccs.   It was saturated with a little quinhydrone so as to leave some of it undissolved.   The sap was then used for the pH determination.

The determinations of isoelectric points were made according to the method of Pearsall and Ewing (1926).   Though this method is not accurate it is adopted in absence of any other method which can be applied to plant tissues.   Equal volumes of roots, stems and leaves were used for each determination.   All determinations were made in triplicates, and mean of the three was taken.   Burettes, pipettes and weights were standardised before use.   Distilled water free from carbon dioxide was used.   Seeds were sown on the 20th June, 1934.   The pH and the I.E.P. of the proteins of the tissues of the roots, stems of the vegetative and the fruiting branches, of the leaves of the vegetative and fruiting branches, of the unshed bolls and bolls were determined.

All the apparatus for these determinations were taken from the Botany Department of the Royal Institute of Science, Bombay, with the kind permission of the Principal, to Surat and they were fitted up in a small room adjoining the fields in the Farm area with no little difficulty.   It was after great labour and time that the delicate pieces of the apparatus were

properly adjusted for work. The large quantities of distilled water required for work were obtained from the Royal Institute of Science, Bombay, at regular intervals, as no distilled water in large quantities is available in Surat. This was found to be the cheapest way of getting distilled water.

It was necessary to keep the records of the changes in the weather factors during the development of the cotton plant as they may be useful in interpreting the results obtained in this investigation. The daily records of humidity, temperature and rain-fall are kept at Agricultural Farm, and so these records were obtained from the Farm.

The percentage of humidity is higher at night than during the day. During the period from June to September the humidity is very high, and becomes low during the rest of the period except when it rains. So during vegetative phase the cotton plant is growing under very humid conditions while during the reproductive phase it is living under less humid conditions.

The maximum temperatures (averages) fluctuate between 82°F. and 96°F. So the cotton plant is not exposed to very high temperatures (averages) (although the individual maximum temperature may go as high as 103°F.). The minimum temperatures (fortnightly averages) fluctuate between 56°F. and 81°F. So the range of variation is greater in this case.

During the vegetative phase of the plant the rainfall is heavy while during the reproductive phase very little rain falls except in the month of November, which is not a usual occurrence. The last showers generally fall in October.

The study of the weather data shows that the cotton plant in its vegetative phase is exposed to moist conditions with adequate rainfall and high humidities and more or less uniform temperatures during the day, fluctuating within very narrow limits. During the reproductive phase the reverse is the case. The plant is exposed to low and very varying conditions of humidity and very wide variations of temperature during the day. It is possible that the first set of conditions is favourable for the vegetative growth and the second set of conditions for the reproductive growth. Any unusual variations in these two sets of conditions may adversely affect the growth of the plant.

As far as the weather conditions that prevailed during the period of this investigation they were normal except for the rains in the first week of November. As there was very little rain in October the showers in November had a favourable effect on the growth of the plant.

The changes in the pH value of the roots

When the seeds germinate the pH value of the sap of the roots is near the neutral point but the sap becomes more and more acidic as the growth of the seedlings proceeds. This rise in acidity may

be due to the formation of acidic substances in the decomposition of the reserve products or in the regeneration of proteins. In August the pH value of the sap is very low. Later the cell sap of the roots becomes again less and less acidic as indicated by the rise in the pH value till October, when once again the pH value shows a fall. At the end of December and in January the sap becomes less and less acid and reaches a value of pH 6·44. The sap then again becomes acidic till the lowest pH value is reached in March when the plant's activity terminates. Thus the pH values of the sap show rise and fall in acidity five times during the life cycle of the plant (1) Increase in acidity from July to August (2) Decrease in acidity from September to October (3) Increase in acidity in November and upto the middle of December (4) Decrease in acidity in December-January (5) Final increase in acidity till the plant reaches its stage of senescence (Fig. 1).

Changes in the pH value of the vegetative branches

The changes in the pH value of the vegetative branches are similar to those obtained in the case of the pH of the sap of the roots except that the rise and fall in the pH of the stem are observed a week or ten days later than in the case of the roots. The changes in the pH of the roots precede the changes in the pH of the stem. The sap of the stem also shows fall and rise in the pH five times during the season (Fig. 1).

Changes in the pH values of the fruiting stems (branches)

It was considered of interest to determine the pH value of the sap of the fruiting stems to study if any differences exist in the reaction of the sap between the vegetative and fruiting stems (Fig. 1). The fruiting stems were taken from September for these determinations. It was found that the changes in the pH of the sap of the fruiting stems do not differ from the corresponding changes in the pH of the vegetative branches. The pH values of the fruiting branches are lower than the pH values of the sap of the vegetative branches except in the last stages of growth.

The changes in the pH of the leaf (vegetative branch)

The acidity of the cell sap increases upto the middle of July. Afterwards the acidity shows no rise or fall for a month and a half. The acidity of the sap of the leaves decreases from September and it continues to decrease till January, when the cell sap becomes almost neutral in reaction. Then there is again a sudden increase in acidity as in the case of roots and branches when the plant reaches its senescent stage. No depression in the pH value (increase in acidity) is noticed in December as was the case in the pH of the stem and roots (Fig. 1).
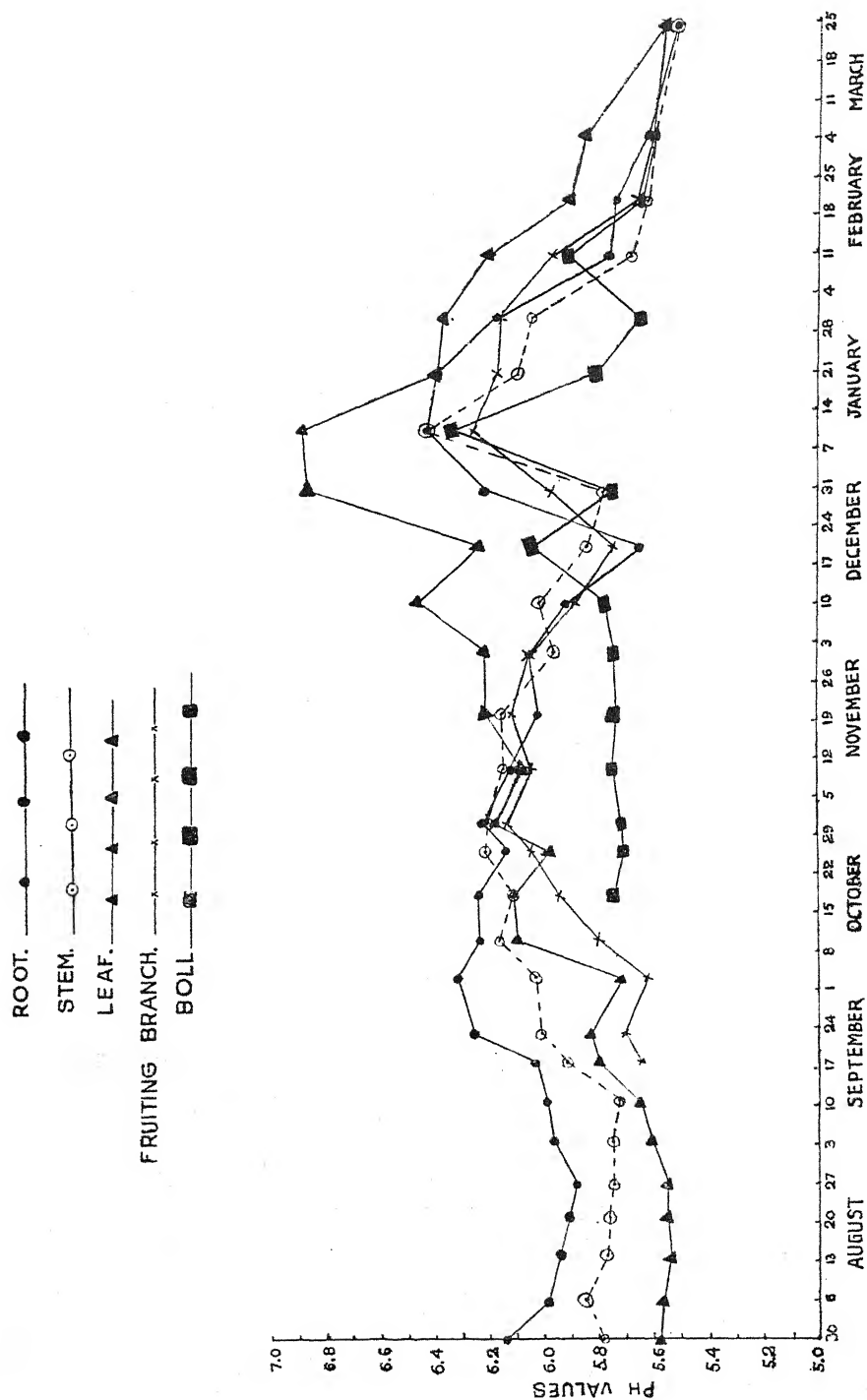
Fig 1

The changes in the pH of the leaf (fruiting branch)

The pH of the sap of the leaves from the fruiting branches is also determined separately but no differences in the fluctuations of the pH of the sap of the leaves of the fruiting branches as compared with the pH of the sap of leaves from the vegetative branches are noticed (Fig. 1).

The pH values of the roots, stem and leaves

During the early stages of growth the pH of the sap of the roots is highest, that of the sap of the stem medium, and that of the sap of the leaves lowest indicating a fall in the pH from the roots towards the leaves. The sap of the leaves is more acidic than that of the stem and the roots, and the sap of the stem is more acidic than that of the roots. These relations between the pH values of the roots, stem and leaves are noticed till November. During the later stages of growth, from November onwards the sap of the leaves is less acidic than that of either the roots or stem and in March the pH of the saps of all organs of the plant is the same.

The sap of the branches is more acidic than the sap of the roots till November but later on these relations are not maintained.

The changes in the pH of the fruiting branch, fruiting-branch-leaf and bolls

The pH of the sap of the leaves of the fruiting branches, is, except in the beginning, constantly higher than that of the branch itself. The sap of the fruiting branches is more acidic than that of the leaves. This is a remarkable feature for in the case of the leaves of the vegetative branches these relations are in a reversed order as the sap of the leaves is more acidic than that of the stem. In the case of the unshed bolls the sap is more acidic than that of the stems (branches). There is no significant difference between the pH values of the shed and unshed bolls (Fig. 1).

The pH value of the sap of the tissues of the roots, stems (branches) and leaves of the whole plant reaches its maximum during the intense flowering period as well as in the successful bolling period in December-January. The cell sap at that period is the least acidic. The rise in the pH value is abrupt and steep. How this sudden rise in the pH is correlated to the intense flowering period or the successful bolling period is difficult to say.

Isoelectric point of the proteins of the roots, stems (branches) and leaves

The results of the isoelectric point of the proteins of the roots show wide fluctuations. Such changes in the values of the pH of the isoelectric point of a protein are not expected. It is extremely

unlikely that the isoelectric point of the protein of an organ of a plant would change. But it is possible that the apparent isoelectric point of a protein might change if the salt concentration of the tissue is altered and therefore these differences in the isoelectric points of the proteins of the roots are not due to changes in the gross chemical composition of the proteins but are due to the alteration in the ionic concentration of the tissues of the roots. It is therefore more accurate to term these values of the isoelectric point of the proteins as the values of apparent isoelectric point. It is quite probable that the ionic concentration of the tissues of the roots may vary from week to week and from one part of the root to the other and therefore the apparent isoelectric point of the tissue may also vary (Fig. 2).

As the results stand the value of the apparent isoelectric point of the roots in the earlier stages of growth is less than that of the apparent isoelectric point of the roots in the later stages of growth. The chlorine ions diffuse more rapidly during the earlier stages of the growth of the roots than in the later stages. Therefore the tissues of the roots are more electro-positive in the earlier stages than in the later stages of growth (Fig. 2).

In the case of the stems (vegetative and fruiting branches) the values of the isoelectric points do not show such wide variations as in case of the roots. As in case of the roots, the values for the apparent isoelectric point of the stems are highest at the termination of the plant's activity and there is not much difference between the pH of the isoelectric points of the roots and stems.

In the case of the leaves the apparent isoelectric points of the leaves show small differences and the values rise towards the end of the growth cycle (Fig. 2). In the month of March the isoelectric point is the same in the roots, stems and the leaves.

During the vegetative growth period the proteins of the tissues of the roots and stems and leaves are more electro-positive than during flowering and successful bolling stage. When the fruiting activity terminates the proteins of the tissues of the roots, stems and leaves reach their true isoelectric point on account of the protoplasmic hysteresis. Thus the true isoelectric point of the proteins of the cotton plant is probably somewhere between $5 \cdot 2$ and $5 \cdot 3$. In the case of the roots that value of the isoelectric point is obtained earlier than in the stems and leaves.

If $5 \cdot 2$ to $5 \cdot 3$ be taken as the true isoelectric point of the plant, the roots and stem are distinctly more electro-positive during the whole life of the plant, than the leaf as in the latter the value of the isoelectric point goes beyond $5 \cdot 3$ in the month of February. Probably on account of this reason the proteins of the roots allow water more readily to diffuse into stem and the stem allows water more readily to diffuse towards the leaves.
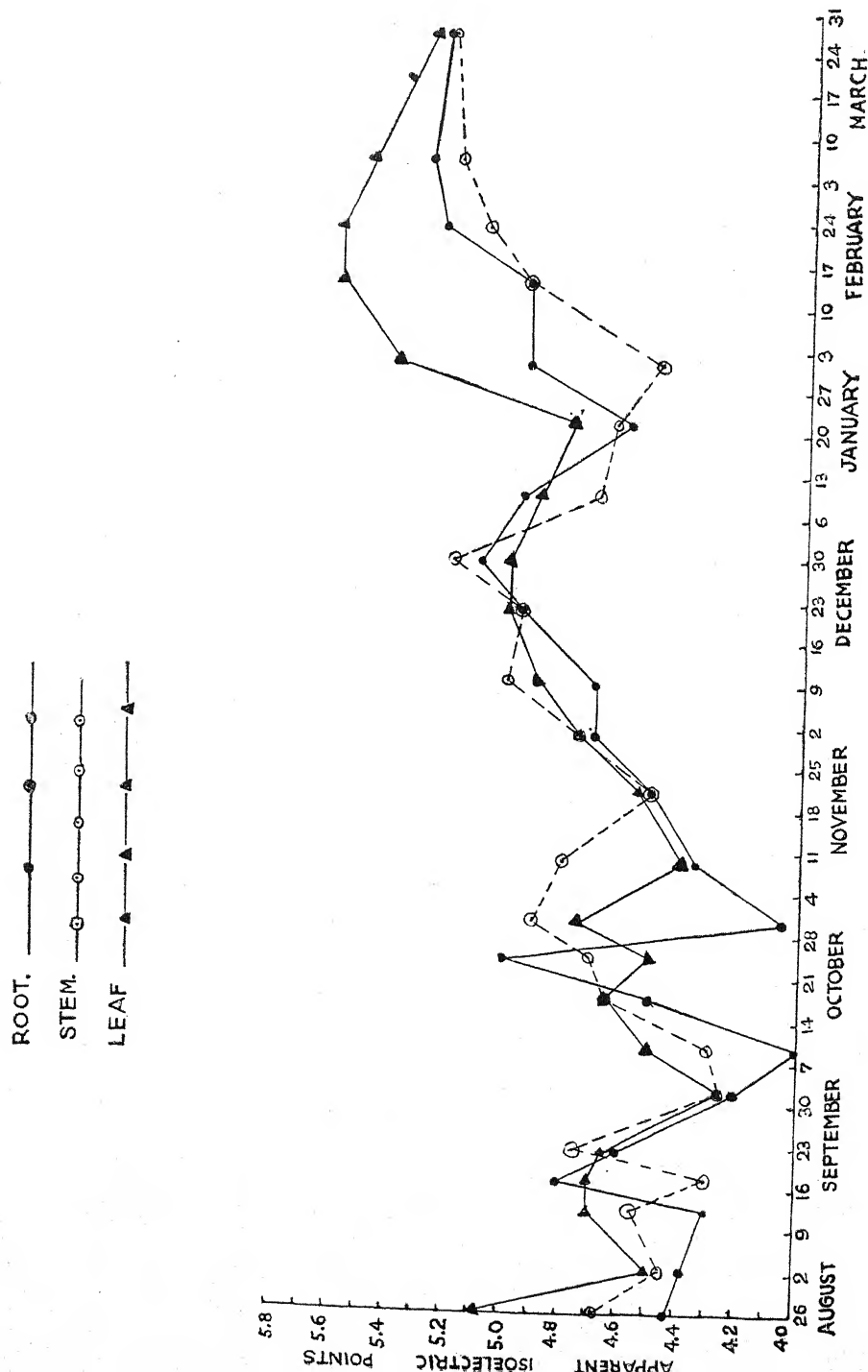
Fig 2.

The lower values of the apparent isoelectric point of the root as compared with those of the apparent isoelectric point of the stem and leaf indicate that the chlorine ions (anions) are allowed to diffuse more readily through the roots, than through the stem or leaves and for this reason it is surmised that the proteins of the roots are more electro-positive than those of the stems and leaves.

The changes in the charge on the proteins of the tissues of the roots, stem and leaves are although important, the reaction of the medium of the cells of the tissues in which the proteins are placed is a still more important factor, as the combining power of the proteins with the basic and the acidic ions depends also on the hydrogen ion concentration of the solution in which they are placed. If the hydrogen ion concentration of the medium is the same as the pH of the isoelectric point of the proteins the latter lose their amphoteric properties and will neither act as bases nor acids. An ampholyte placed in a weak buffer mixture changes towards greater alkalinity according to the reaction of these buffer mixtures which are more acidic than the isoelectric point, and changes towards greater acidity according to the reaction of those buffer mixtures that are more alkaline than the isoelectric point. This is because the ampholyte acts as an acid in solutions more alkaline than the isoelectric point and as an alkali in solutions that are more acidic than the isoelectric point. At the isoelectric point the reaction of the solution in which the ampholyte is placed remains unchanged.

In view of these known ways of the behaviour of the proteins it would be interesting to study the results of the isoelectric point of the proteins of the roots, stem and leaves of the cotton plant in relation to the hydrogen ion concentration of the solutions in which they are placed. The pH value determination of the cell sap of the roots, stem and leaves gives an adequate and reliable information about the medium in which the proteins are placed and therefore it is possible to infer the physical behaviour of the proteins of the roots, stem and leaves of the cotton plant during the different stages of its growth.

It was stated above that the reaction of the solution in which the ampholyte is placed remains unchanged at the isoelectric point of the ampholyte. It is also known that the proteins are at their isoelectric point when the protoplasmic hysteresis takes place. This has been found to be the case in the rice plant by Dastur and Kalyani (1934) that at the termination of the activity of the tissues the proteins are at their isoelectric point. Now in this investigation the isoelectric point of the ampholytes of the tissues of the cotton plant lies between 5·2–5·3. This is the value when the plant tissues reach the end of their functional activities in March. The pH value of the cell sap of the tissues of the same organs of the cotton plant is 5·5 (average value) in the month of March. This value is very near the value of the isoelectric point of the tissues. Thus the

investigations support the conclusions arrived at about the physical properties of the proteins in *in-vitro* experiments that at the isoelectric point the reaction solution remains unchanged and the pH of the solution is near the isoelectric point of the proteins. Thus the termination of the functional activity is accompanied by the inactivation of the ampholytes as the latter lose their amphoteric nature among other physical properties.

Now taking the roots separately the apparent isoelectric point of the roots on 26th August is 4·42. If the true isoelectric point of the proteins is 5·25, evidently the proteins are positively charged. Secondly the pH value of the cell sap is 5·83 on the same date when the plants are 64 days old. If the values of the pH of younger plants are studied the cell sap is still less acidic (pH 6·21– 6·05). So the proteins of the roots are in an alkaline medium and will naturally behave as acids. As they behave as acids, they combine with bases more readily than with acids. As they combine with bases they render the solution in which they are placed more and more acid. This has been actually found to be the case as the sap of the roots increases in acidity (pH falls) from 10 days' stage upto 70 (64) days' stage of the plant. As the reaction of the cell sap is rendered more acidic than before, the proteins begin to combine more rapidly with the acids rather than with the bases and there is a rise in the pH value of the sap. Thus the behaviour of the proteins is again very much towards the alkaline side of the isoelectric point. The same process is again repeated and the pH of the cell sap falls once more to rise again in January when the plants are 192 days old. In February there is a fall in the pH value till the pH of the sap is brought nearer to the isoelectric point in March. Thus the periodic fall and rise in the pH (*i.e.*, the increase and decrease in the acidity) of the cell sap can be explained.

Conclusions

The pH of the cell sap of the tissues of the roots, stem and vegetative branches does not remain constant during the life time of the plant. An increase and decrease in acidity of the sap occurs during the growth of the plant. In the case of the fruiting branches similar behaviour is noticed while in the case of the leaf-tissue-fluids there is a regular decrease in acidity. The root sap is less acid than that of the stem while the stem sap is less acid than that of the leaf upto 31st October. The sap of the bolls is more acid than that of the leaves of the fruiting branches.

The determinations of the isoelectric points of the proteins of the tissues of different organs show that the apparent isoelectric points of those tissues are slightly lower than the true isoelectric point. The difference in the apparent isoelectric point and the true isoelectric point may be very likely due to the changes in the salt concentration of the tissues. As the values of the apparent isoelectric point are lower than the values of the true isoelectric

point, it indicates that the diffusion of anions, the chlorine ions, occurs less rapidly than it should. It means the proteins are electro-positive, *i.e.,* they carry the positive charge.

The proteins of the roots, stem and leaves are placed in the alkaline medium as the pH of the tissue-fluids is on the alkaline side of the isoelectric point. Therefore they act as acids and combine with bases. In the case of the roots and stem the alternate rise and fall in the pH value of the cell sap can be correlated to the behaviour of the proteins. When the proteins combine more readily with the bases than with the acids, the acidity of the sap is increased. When the acidity of the sap is increased the behaviour of the proteins is also altered and they now combine more readily with the acids than before. It is these variations in the charge carried by the proteins that make possible the migration of different ions within the body of the plant. It may also be responsible for the absorption of both positively and negatively charged ions.

At the termination of the plant's activity the proteins reach their isoelectric point; that is they are no longer active and they lose their combining power. This investigation has shown that when the tissues become inactive, their proteins are at the isoelectric point. It is very likely that proteins lose their physical properties when the tissues become inactive, very probably due to the coagulation of protoplasm.

Summary

There is an alternate increase and decrease in acidity of the sap of roots and stem taking place five times during the season. In the case of the leaves after the first increase in acidity there is a regular decrease in acidity till the last stage when the plant shows signs of drying up. A big fall in the acidity of the sap occurs during the intense flowering period.

The proteins of the tissues of the plants are on the alkaline side of the isoelectric point during the entire growing period of the plant. The proteins are positively charged. As the reaction of the sap changes during the growth, the positive charge on the proteins increases or decreases. Thus the number of protein anions and cations changes as the pH value changes and *vice versa*. These variations in the charge of the proteins may be responsible for the absorption of the positively and negatively charged ions as at one time proteins combine more readily with bases than the acids and at other times less readily with bases than with acids.

Literature Cited

CLARK, W. M. (1920). Determination of Hydrogen ion concent-ration. William & Wilkins Co.

DASTUR, R. H. & KALYANI, V. V. (1934). Hydrogen ion concentration and the intake if nitrogen by the rice plant. *Ind. Jour. Agri. Sci.*, Vol. IV. VI Part V.

HAWKINS, R. S., CLARKE, S. E., SERVISS, G. H. & HOBART, C. A. (1934). Varietal differences in cotton boll shedding as correlated with osmotic pressure of expressed tissue fluids. *J. Agri. Res.*, 48, 149.

INGALLS, R. A. & SHIVE, J. W. (1931). Plant Physiology, William & Wilkins Co., 6 : 1–203.

PEARSALL, W. H. & J. EWING (1924). The diffusion of ions from living plant tissues in relation to protein isoelectric points. New Phytologist, 23 : 4.

Acknowledgment

GAMETOGENESIS AND EMBRYOGENY IN *LOBELIA NICOTIANÆFOLIA* HEYNE.

BY

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The literature on the Lobeliaceae and the allied family Campanulaceae has been reviewed by Schürhoff (1926) and Schnarf (1929, 1931) and recently in a previous paper by the present author (1935). A paper by Rosen (1932) which had escaped notice previously may however be mentioned here. In this paper a number of plants belonging to the Campanulaceae and a few species of *Lobelia* have been dealt with and the modes of endosperm formation and the separation of haustoria are described at length, in addition to the recording of the chromosome numbers in the plants studied.

The present paper deals with *Lobelia nicotianaefolia* Heyne and includes a survey of the more important stages of meiosis in the microspore mother cells, gametogenesis and embryogeny. The chromosome number is reported here for the species for the first time.

Material and Methods

Lobelia nicotianaefolia Heyne is a tall herb reaching a height of six to seven feet and bearing large leaves and a dense terminal raceme of conspicuous flowers. The material was collected along the banks of a small stream at Koppa, Mysore State, during October 1936 and fixed in Bouin's killing fluid. The sections were cut from 10μ to 14μ and stained in Heidenhain's iron-alum haematoxylin.

Meiosis in the Microspore Mother Cells

The reticulum of the mother cell nucleus resolves itself into a number of pachytene threads which are made up of two longitudinal units (Plate I, Fig. 1). Armand (1921) states that the threads are undivided longitudinally in the preparations of the

nuclear cavity in all directions (Plate IV, Fig. 2) and each thread very soon begins to form a number of twists all along its length to establish the chiasmata (Plate IV, Fig. 3). The chiasmata are gradually reduced in number to a minimum and are finally terminalised when the bivalent chromosomes are formed. The chromosomes are extremely condensed and stain deeply. With the disappearance of the nuclear membrane and the formation of the spindle fibres, the chromosomes are separated and drawn to the poles of the spindle (Plate IV, Fig. 5).

During the second division in the mother cells, there is a prominent zone of large densely staining granules between the two spindles (Plate IV, Fig. 6). The separation of the microspores takes place by advancing peripheral furrows (Plate IV, Fig. 7). The microspores acquire the characteristic two layered thick wall and become spherical to form the pollen grains.

The pollen grain nucleus divides while the grains are still within the anther loculus. At the time of dehiscence of the anther, each grain has a large tube nucleus and two small male cells (Plate IV, Fig. 8).

During the study of meiosis in the microspore mother cells, the number of bivalent chromosomes was determined to be fourteen (Plate IV, Fig. 4). Armand (1912) mentions that the haploid number of chromosomes is eight in the species that he studied.

Megasporogenesis

The two-carpelled inferior ovary has a central placenta which forms a number of lobes bearing the innumerable anatropous ovules (Fig. 1). Each ovule arises on the placenta as a nucellar
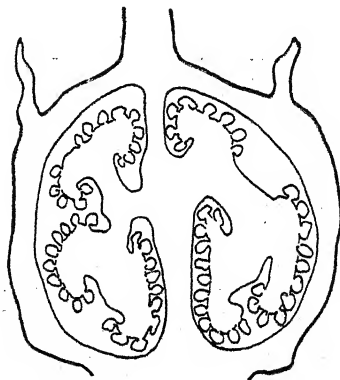
Text fig. 1. Longitudinal section of the ovary to show the lobes of the placenta bearing the innumerable anatropous ovules.

primordium, and at quite an early period of its growth, forms a ring of cells which becomes the single integument. There is a single large hypodermal cell at the tip of the nucellus (Plate IV, Fig. 9). This functions directly as the megaspore mother cell and after undergoing two successive divisions gives rise to the linear tetrad of megaspores. The chalazal megaspore enlarges in size and forms the embryo-sac (Plate IV, Fig. 10). Sometimes the micropylar megaspore develops into the embryo-sac instead of the usual chalazal one (Plate IV, Fig. 11).

Soon after the disorganisation of the upper three megaspores of the linear tetrad, the nucellar cells which form a single investing layer at the region of the growing embryo-sac begin to break down (Plate IV, Fig. 12 and Plate V, Fig. 13). As a result of this, the innermost layer of the integument which becomes conspicuous on account of regularly arranged tabular cells with rich contents comes in direct contact with the sides of the embryo-sac and forms the integumentary tapetum. The tapetum is the chief source of nutrition for the embryo-sac during its period of formation and growth. Its function as a nutritive jacket ceases with the formation of the micropylar and chalazal haustoria after fertilisation and in the mature seed, it persists as a layer of thick walled cells to give additional protection to the developing embryo.

The surviving megaspore of the linear tetrad gives rise to the typical eight-nucleate embryo-sac (Plate IV, Fig. 12 and Plate V, Figs. 13–17). The synergids are organised very early and in the fully developed embryo-sac become extremely long with a tapering anterior end and a broad vacuolate posterior end. The antipodals are formed into definite cells with the lower ends pointing and are fairly conspicuous for a time, after which they disorganise (Plate V, Figs. 16, 17). The two polar nuclei which migrate to the centre of the embryo-sac are usually pressed against each other on coming into contact and when the egg is ready for syngamy, fuse to form a large fusion nucleus (Plate V, Figs. 15–17). A similar condition is also observed in *Lobelia trigona* (Kausik, 1935) and *L. syphilitica* (Ward, 1880), but in *L. erinus, L. urens* and *L. Dortmanna,* the two polars merely lie close together according to Armand (1912).

The behaviour of the antipodal end of the embryo-sac in *L. nicotianaefolia* Heyne deserves special mention. At the time of the commencement of the formation of the eight nucleate embryo-sac (Plate V, Fig. 14), it begins to grow past the antipodals as a small process penetrating through the mass of the rich chalazal cells which are nutritive in function and acts as a haustorial organ for the developing embryo-sac (Plate V, Figs. 16, 17). The antipodal cells with their pointed lower ends also seem to take part in the conduction of the material thus obtained by the antipodal process of the sac. The chalazal tissue is however not completely destroyed by the antipodal process, because most of it persists and

later when the embryo develops and with the formation of a prominent chalazal haustorium, it constitutes an important nutritive tissue in the ovule.

Endosperm and Haustoria

The endosperm is cellular from the beginning and is formed soon after fertilisation. When it is fully formed, it completely occupies the distended embryo-sac which fills the entire cavity of the seed within the integument. In the two terminal regions of the embryo-sac the endosperm forms two prominent haustoria, the micropylar and the chalazal, which are important organs for the absorption of nutrition from the micropylar and chalazal nutritive tissues respectively.

The micropylar haustorium is very large and consists of two elongated cells, each with a single large nucleus (Plate V, Fig. 18). The haustorium stains very deeply on account of its rich contents absorbed from the nutritive cells situated in the integument at the region of the micropyle.

The chalazal haustorium is also equally conspicuous and consists similarly of two uninucleate cells (Plate V, Fig. 19). The haustorium penetrates through the chalazal nutritive tissue whose cells consequently lose their rich contents and get crushed by the haustorium, the distal ends of the two cells of which are spread out as large expansions.

The haustorial function stops after a time when the embryo is fully formed and the remnants of the haustoria are seen in the mature seed as darkly staining shrivelled up masses in various stages of disintegration.

Embryo

The fertilised egg, after a series of transverse divisions, gives rise to a filamentous pro-embryo in which are seen a long suspensor of a variable number of cells and the embryo proper, primarily made up of four large cells arranged in a row (Fig. 2). The first two cells of the suspensor immediately following the embryonal cells are also fairly conspicuous and contribute to the completion of the basal region of the embryo. The first two cells of the row of four embryonal cells are the earliest to divide by two vertical walls at right angles to each other (Fig. 3), followed by the formation of a single vertical wall in the third cell also (Fig. 4). The embryo therefore consists at this stage of tiers of cells, the first two tiers with four cells each and the third with two, while the last cell is still undivided. The first tier of cells undergoes the next division by two oblique walls, one on each side of the first vertical wall (Fig. 5) and the further divisions are by periclinal walls in all the tiers of the embryo to form the dermatogen (Fig. 6). The central mass of cells within the dermatogen in the second tier of cells constituting the hypocotyl gives rise, after a few more divisions,

to the plerome and the periblem (Figs. 7–9). The body regions in the embryo may all be traced to the four primary embryonal cells— the first cell giving rise to the cotyledons and the stem tip, the second constituting the hypocotyl and the third and the fourth forming the hypophysis and the radicle respectively (Fig. 9).
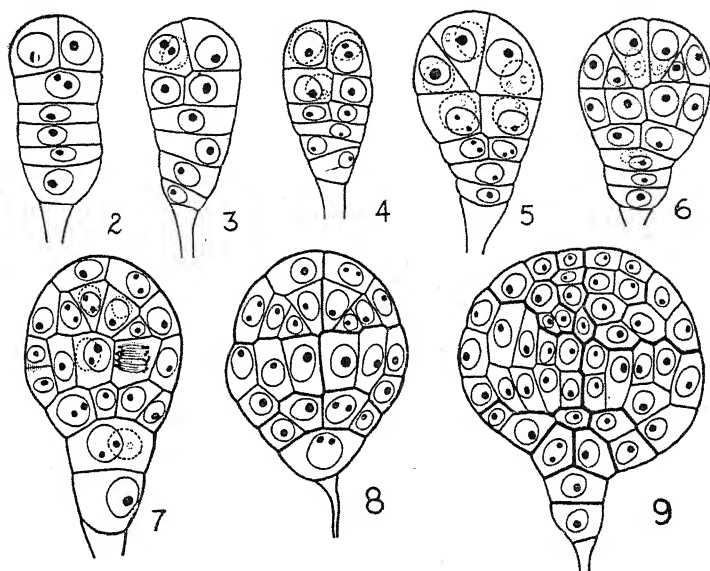
Text figs. 2—9. Stages in the development of the embryo; The primary walls are clearly seen in figs. 8 & 9. All ×900.

The development of the embryo in *L. nicotianaefolia* Heyne is similar to that in *L. trigona* in many respects, but the character- istic constrictions; which are a conspicuous feature in the filamentous pro-embryo of *L. trigona* are absent in the species studied here.

The embryo is completely formed in the mature seed and develops at the expense of the endosperm. The seed has a hard coat, the outermost layer of which has cells with extremely thickened walls. The innermost layer of cells which before fertilisation functioned as the tapetum also has thick walls and therefore gives added strength to the seed coat.

Conclusions

The supplies of nutrition for the growing embryo-sac at differ- ent periods and the formation of devices for the absorption of materials for the developing embryo from parts of the ovule adjacent to the embryo-sac are too well known in many families of the Sympetalae to need an elaborate discussion here. In the case

of *Lobelia trigona* studied previously by the present author, mention has already been made of the partial breaking down of the chalazal tissue by the antipodal end of the embryo-sac prior to fertilisation. In the case of the species studied here a similar state of affairs also exists. The antipodal end of the embryo-sac here becomes more pronounced in forming a process which grows beyond the antipodals into the chalazal nutritive tissue and forms an embryo-sac haustorium, which is later replaced by the aggressive chalazal haustorium. The antipodals which persist for some time share in the absorption and conduction of materials thus obtained by the antipodal process of the sac. They begin to disorganise only when the embryo-sac is fully organised and is ready for fertilisation. The period that elapses between the commencement of the formation of the embryo-sac and the actual time of fertilisation seems to be fairly long, during which time the embryo-sac is storing plenty of nutritive materials obtained from the chalazal tissue.

The nutritive tissue at the micropylar region of the integument is less pronounced in the earlier stages. It becomes conspicuous with rich cell contents only when the embryo is about to be formed and the micropylar haustorium is differentiated. Both the micropylar and the chalazal haustoria are aggressive structures drawing nutrition from the two nutritive tissues, where the cells consequently become crushed and large cavities are formed in later stages containing the remnants of the haustoria.

Apart from the two haustoria which form the chief organs for obtaining nutrition for the embryo, the tapetal jacket formed by the innermost layer of cells of the integument is an important source of nutrition for the embryo-sac during its period of formation and growth. It becomes a protective layer only later when the seed is formed and thus has two different functions at different periods in the history of the embryo-sac, as a nutritive jacket before fertilisation and as a protective layer when the embryo is formed.

Summary

1. The salient features in the life history of *Lobelia nicotianaefolia* Heyne are described.

2. The important stages in the meiosis of the microspore mother cells are followed and the haploid number of chromosomes is recorded to be fourteen for the species.

3. The different stages in the development of the embryo-sac, the presence of an antipodal process of the embryo-sac for the absorption of nutrition and the formation of the haustoria after fertilisation are discussed.

4. The development of the embryo is studied in detail and the relation of the body regions of the embryo to the primary embryonal cells is traced.

The author's sincere thanks are due to Dr. M. A. Sampath-kumaran, M.A., Ph.D., University Professor of Botany, University of Mysore, to whom he is greatly indebted for direction and encouragement during the progress of this work.

Literature Cited

ARMAND, L. (1912). Fecundation et developpement de l'embryon chez Lobéliacées. *Compt. rend. Acad. Sc.*, 155, Pp. 1534–1536.

————(1921). Les phénomènes nucleaires de la cinèse heterotypique chez le *Lobelia urens* et quelques Campanulacées. *Ibid.*, 172, Pp. 762–764.

KAUSIK, S. B. (1935). The Life History of *Lobelia trigona* Roxb. with special reference to the nutrition of the embryo-sac. *Proc. Ind. Acad. Sc.*, 2, Pp. 410–418.

ROSEN, W. (1932). Zur Embryologie der Campanulaceen und Lobeliaceen. *Meddel. Goteborgs Bot. Trad.*, 7, Pp. 31–42.

SCHNARF, K. (1929). Embryologie der Angiospermen. Berlin.

————(1931). Vergleichende Embryologie der Angiospermen. Berlin.

SCHURHOFF, P. N. (1926). Die Zytologie der Blütenpflanzen. Stuttgart.

WARD, H. M. (1880). A contribution to our knowledge of the embryo-sac in Angiosperms. *Journ. Linn. Soc., Bot.*, 17, Pp. 519–546.

Explanation of Plates IV and V

PLATE IV

Figs. 1–7 ×2700; rest ×900

Figs. 1 and 2. Nucleus of the microspore mother cell during early and late pachytene showing the double nature of the threads.

Fig. 3. A later stage; the formation of twists in the threads is clearly seen.

Fig. 4. A metaphase plate showing fourteen bivalents.

Fig. 5. Early anaphase to show the separation of the chromosomes.

Fig. 6. The spindles for the second division: note the zone of granules between the spindles.

Fig. 7. The formation of peripheral furrows between the daughter nuclei.

Fig. 8. Pollen grain at shedding stage showing the large tube nucleus and two small male cells.

Fig. 9. Longitudinal section of a young ovule with the hypodermal archesporial cell.

Figs. 10 and 11. The degenerating megaspores of the linear tetrad: in Fig. 11 is shown a case of the micropylar megaspore enlarging at the expense of the others.

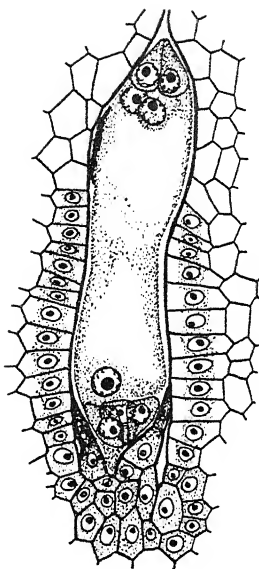Fig. 12. Two nucleate embryo-sac: the nucellus is breaking down.

PLATE V

All figures ×600, except 18 and 19 ×900

Figs. 13 to 17. Stages in the formation of the embryo-sac; in Figs. 16 and 17 the formation of the antipodal process of the embryo-sac is shown.

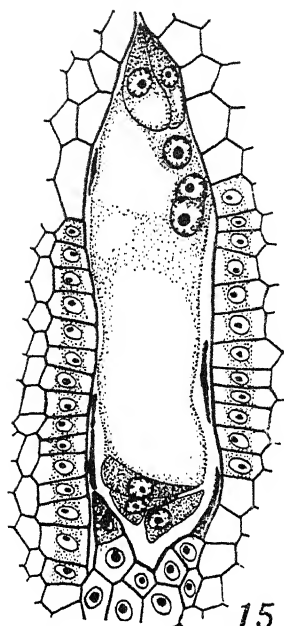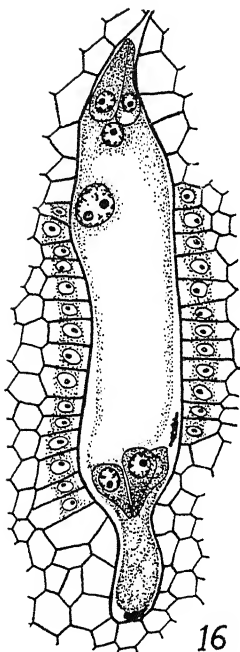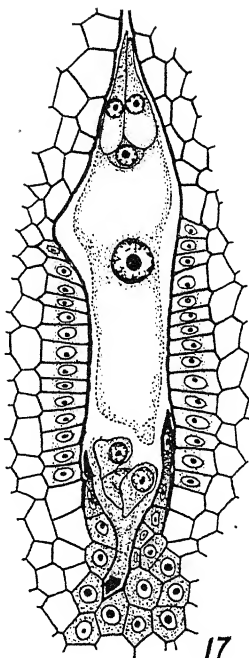Figs. 18 and 19. The micropylar and the chalazal haustoria respectively at the time of their maximum activity.
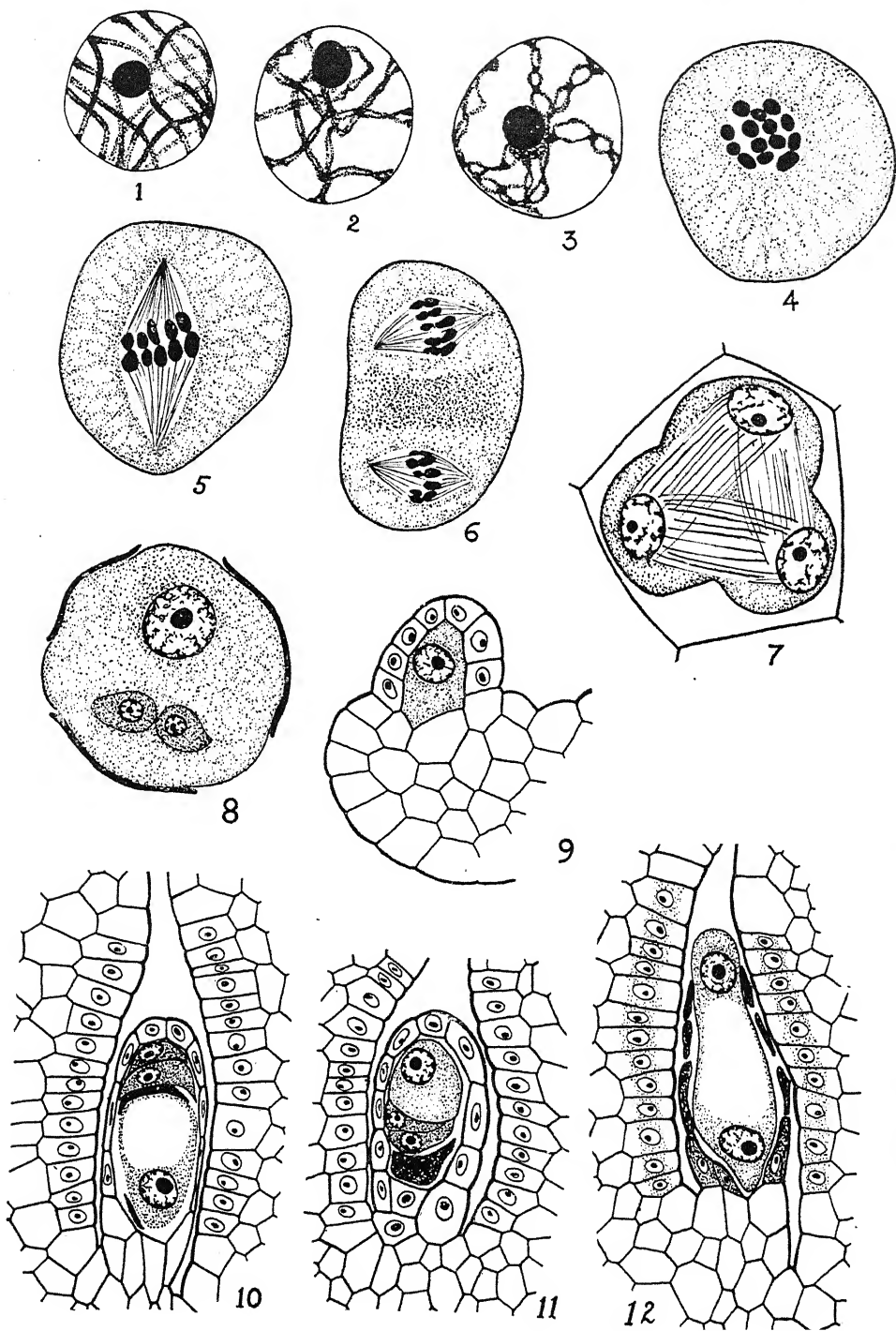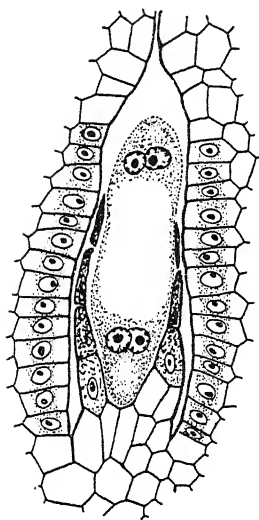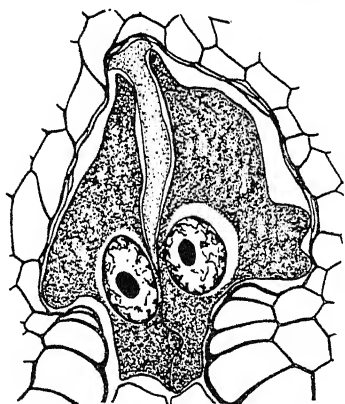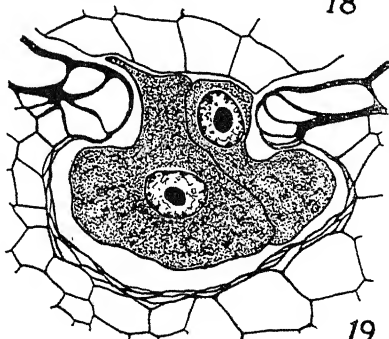
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A NOTE ON THE MORPHOLOGY OF THE GYNAECIUM, OVULE AND EMBRYO-SAC OF *PSORALEA CORYLIFOLIA* L.

BY

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Recently the writer investigated the development of the carpels in the family Menispermaceae (Joshi 1937 *a* and *b*), and presented evidence for reduction in the number of ovules in the carpels of this group during the course of its evolution. Following this investigation, it was decided to see if other groups of flowering plants with uni-ovulate carpels also yield similar evidence. Most members of the family Leguminosae possess many-ovuled carpels. There is, however, a fairly large number in which the carpels are only a few-ovuled or even one-ovuled. A list of such forms has been given by Thompson (1929, p. 41). The family is thus eminently suitable for such study, and the morphology of *Psoralea corylifolia* Linn. has been investigated as a representative of the one-ovuled forms in the Papilionatae.

When the investigation was started, there was no desire to study the embryo-sac. The material microtomed for the other purpose, however, also showed on examination the different stages in the development of the embryo-sac. A brief account of this, therefore, is also included here.

The Gynaecium

The gynaecium in *Psoralea corylifolia* does not at all show the dual symmetry (Figs. 1 and 2), which Saunders (1929) regards characteristic of some Leguminosae. Its vascular system is also that of a single carpel, there being two marginal bundles and one dorsal or midrib bundle (Fig. 2). Although at the base of the gynaecium there are a few extra vascular strands—remnants of the axial vascular system—but these soon come to an end. There are thus no grounds for regarding the gynaecium of *Psoralea corylifolia* to be composed of two carpels as believed by Saunders to be characteristic of Leguminosae. It is, however, unnecessary to take up this question in detail here, as Moore (1936) has only recently dealt with it very fully and the writer completely agrees
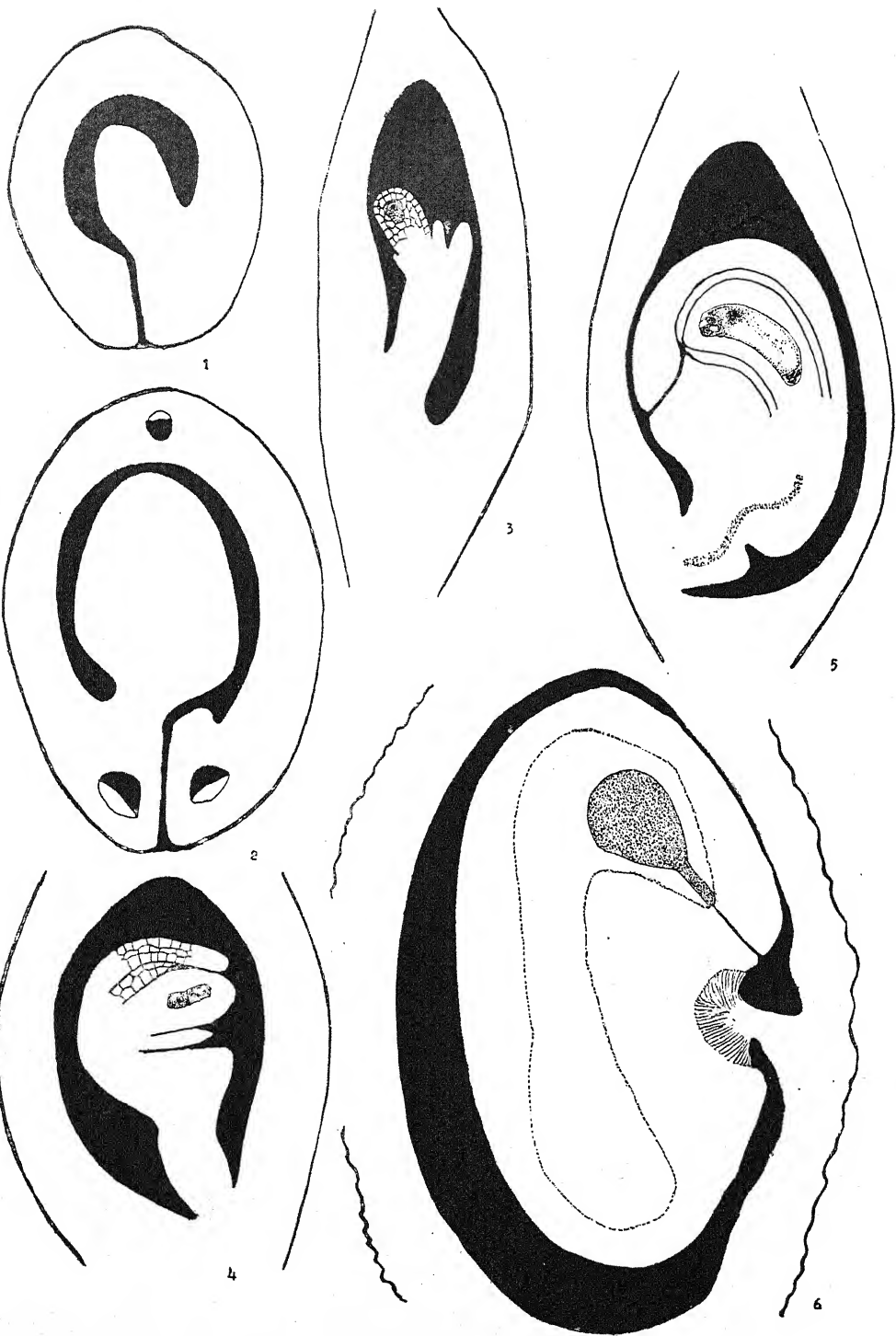
with his views. Similarly, there seem to be no strong grounds for interpreting the legume as a phylloclade-like structure, as done by Thompson (1929). The evidence from comparative morphology is still largely in favour of interpreting the legume as equivalent to a single foliar carpel, as was done long ago by Goethe (1790).

The Ovule

Although the carpel of *Psoralea corylifolia* possesses a single ovule, it is still marginal in its attachment. It may arise from the left or the right margin of the carpel (Figs. 1 and 2). Further the placenta on the other margin from which the ovule is not arising is often equally developed as the placenta on the ovule-bearing margin. This supports the view that the single-ovuled condition of the carpel in this species has resulted by reduction from a more-ovuled state.

The young ovule at first grows straight, but as it comes in contact with the dorsal wall of the carpel, its straight growth is checked and it bends upwards (Fig. 3) just as Reeves (1930) has noted in *Medicago*. Next it becomes somewhat amphitropous (Fig. 4). The form of the ovule at the mature embryo-sac stage (Fig. 5) is very peculiar. It has features of both amphitropy and anatropy, but it is also not far removed from the atropous condition. Further it is broader towards the micropylar end and narrow towards the chalazal end. This peculiar half-ana-amphitropous form of the ovule may be regarded to have originated as a result of reduction in the number of ovules in the carpel. If there had been another ovule growing above this, that would have pressed it downwards, and it would have assumed an anatropous form. In the absence of another ovule, it meets with no pressure from above, and consequently retains the unusual form which has been described above.

After fertilisation, as the embryo is developing the funicle of the ovule expands at its apex. The form of the ovule thus undergoes another transformation. It becomes nearly campylo-tropous (Fig. 6).

The ovule possesses a small amount of nucellus and two integuments. Both the integuments arise nearly simultaneously, or the inner differentiates a little before the outer. The outer, however, soon overtakes the inner (Fig. 4), and is chiefly concerned in the formation of the micropyle (Fig. 5). At the time of the tetrad formation, the inner integument consists of two layers of cells and the outer of three layers (Fig. 4). The inner does not undergo any increase in thickness after this stage, while the outer becomes greatly developed (Fig. 5).

Figs. 1 and 2. Transverse sections of two young carpels showing the attachment of the ovules and the vascular system. ×180.

Figs. 3–6. Longitudinal sections of carpels showing the form of the ovule at various stages of development: Fig. 3, at the megaspore-mother cell stage; Fig. 4, at the tetrad stage; Fig. 5, at the mature embryo-sac stage; Fig. 6, during the development of embryo. Figs. 3 and 4, ×180; Fig. 5, ×85; Fig. 6, ×45.

Development and Structure of the Embryo-sac

There is a single hypodermal archesporial cell, which cuts off a primary wall cell. The latter gives rise to one or two layers of parietal tissue. The megaspore-mother cell gives rise to a linear or T-shaped tetrad of megaspores, of which the chalazal develops into the embryo-sac according to the *normal*-type. The mature embryo-sac shows a normal egg-apparatus, three small antipodals which persist till fertilisation, and two polar nuclei which meet below the egg-apparatus and ultimately fuse there (Fig. 5).

Literature Cited

GOETHE, J. W. VON (1790). Versuch die Metamorphose der Pflanzen zu erklären. Gotha.

JOSHI, A. C. (1937 a). Contributions to the embryology of the Menispermaceae. I. *Cocculus villosus. Proc. Ind. Acad. Sci., B,* V : 57–63.

——————(1937 b). Evidence for reduction in the number of carpels and ovules in the Menispermaceae. *Jour. Bot.,* 75 : 96–98.

MOORE, J. A. (1936). The vascular anatomy of the flower in the Papilionaceous Leguminosae. II. *Amer. Jour. Bot.,* 23 : 349–355.

REEVES, R. G. (1930). Development of ovule and embryo-sac of alfalfa. *Amer. Jour. Bot.,* 17 : 239–246.

SAUNDERS, E. R. (.1929). Illustrations of carpel polymorphism. IV. *New Phytol.,* 28 : 225–258.

THOMPSON, J. McLEAN (1929). Studies in advancing sterility, IV. The legume. *Publ. Hartley Bot. Lab.,* No. 6.

FOSSIL ALGAE FROM WAZIRISTAN

BY

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Communicated by B. Sahni

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Introduction

Two collections of fossils from Waziristan sent by the Director, Geological Survey of India to Prof. B. Sahni, F.R.S., were kindly placed by him in my hands for examination. One of the collections was made by Capt. Murray Stuart from the Takki Zam valley (Waziristan); it contains a few tolerably well-preserved specimens, and is referred by him to the Janjal Plant Series. The second collection, made by Dr. A. L. Coulson from certain localities in South Waziristan, comes partly from the Janjal Plant Series and partly from certain beds referred by the Geological Survey to the Upper Cretaceous. Both the collections are preserved in Calcutta.

From a stratigraphic point of view the specimens are not of much value, but botanically they are of interest as practically no records of algal impressions from the Indian rocks are in existence.

Description

All the specimens described below are from Capt. Stuart's collection from the Janjal Plant Series. The specimens from Dr. Coulson's collection are not included here as they are mostly indeterminable fragments.

Capt. Stuart's collection from the Janjal Plant Series (K 21/169)

The specimens described in this short paper are no doubt algal impressions. Following Seward (1894, p. 4) they are described under the comprehensive generic name *Algites*. The older generic names which suggest relationship with the living forms which cannot be proved, are dispensed with. We know very little of their internal structure or reproductive organs which will help us to determine their affinities. Stopes (1913, pp. 247, 257), however, prefers to describe fossil impressions which suggest Algae under two different genera—*Algites* for flattened impressions, and

Chondrites for forms with a cylindrical thallus. This purely arti-
ficial, though convenient, distinction is better avoided for obvious
reasons. The mode of preservation is often responsible for such
differences in appearance.

It is indeed a hopeless task to try and compare our specimens
with the numerous and controversial "species" of fossil algae pre-
viously described and arrive at a satisfactory conclusion. The
specific identifications tentatively suggested here are to be taken
for what they are worth.

Algites cf. *A. Meyrati* (Fisch.-Oost.)
(Plate VI, Fig. 6)

This small fragmentary specimen is about 3 cms. long and
appears to be irregularly branched (? pinnate). One of the branches
appears to be club-shaped. The branches are of unequal length;
their breadth varies from 2–3 mm. A carbonaceous crust is present.
Maceration of this crust yielded negative results.

I have ventured to compare this specimen with a possible algal
impression described as *Sphaerococcites Meyrati* by Heer (1877,
p. 142; Taf. LVIII. Fig. 9) from the Neocomian of Argentina.
The general habit and size of the branches are comparable with
this form. Stopes (1913, p. 265) considers the species as an alga
of doubtful nature.

Algites cf. *A. intricatus* (Sternb.)
(Plate VI, Figs. 1–4)

This is the commonest species found in these rocks. Frond
delicate, branched, branches irregularly dichotomous and pinnate,
0·25–5 mm. broad. They arise at acute angles and are of unequal
length.

Our specimens can be compared with Heer's (1877, p. 157;
Taf. LXIII, Figs. 1–10) figures of *Chondrites intricatus* from the
Flysch. Though the Waziristan specimens are not quite so complete
as Heer's, they show enough resemblance with the latter in habit,
size and mode of branching to justify a close comparison with it.
The specimens are also closely comparable with *Chondrites
intricatus* figured by Stopes (1913, Pl. I, Fig. 1). The specimens
shown in Fig. 4 probably belong to this species. Their cylindrical
appearance is due possibly to the mode of preservation.

Algites cf. *A. Targionii* (Brongn.)
(Plate VI, Figs. 1 a, 2 a)

Only two specimens were found, associated with *Algites* cf.
A. intricatus. Frond branched, branches irregularly dichotomous
and pinnate, 0·75–1 mm. broad; branching more pinnate than
otherwise.

The specimens can be compared with advantage with Heer's
(1877, Taf. LXIII, Figs. 6 a, 16) figures of *Chondrites Targionii*.

Considering its size, branching and general habit the specimen is closely compared though not specifically identified with it.

Algites sp.

(Plate VI, Figs. 1 *b*, 3 *b*, 4 *b*, 5 *b*)

Thallus straight and unbranched, varying from 0.5 to 2 mm. in breadth, made up of numerous small bladder-like structures, which give it an irregular outline (Pl. VI, Fig. 5*b*).

The specimens are compared in their gross surface features with algal impressions described as *Halymenites lumbricoides* Heer (1877, p. 166; Taf. LXIV, Figs. 11, 12). But the latter is larger in size and its margin appears to be quite regular. Considering the surface features it may be possible to include our specimens within the genus *Halymenites*, if the genus can be retained as such; but since the name suggests affinities that are not proved, it is better avoided. The specimen is described under the comprehensive genus *Algites*. It seems useless to create a new species to accommodate this specimen, as we know very little regarding its structure and affinity. The specimen shown in Fig. 4*b* probably belongs to the same species. Its cylindrical appearance is possibly due to the mode of preservation.

Conclusion

From what has been said above it would be unsafe to depend upon these fossil remains alone for an estimate of the age of the beds from which they come. No doubt the specimens are compared above to species referred to the Cretaceous and early Tertiaries. But as has been stated already their real affinities are obscure. The specimens described above come from the Janjal Plant Series to which Capt. Stuart (1923, pp. 90, 91) assigns a doubtful Jurassic age on the evidence of a distinct lithological resemblance between these fossiliferous limestones and the black Jurassic limestones of the Sheran country.

My thanks are due to Prof. B. Sahni, F.R.S., for helpful criticism. I am indebted to the Director, Geological Survey of India, for the loan of the material and for permission to publish the account in this journal.

Bibliography

HEER, O.—(1877) Flora Fossilis Helvetiae. *Zürich*.

SEWARD, A. C.—(1894) The Wealden flora, Pt. 1. *London*.

STOPES, M. C.—(1913) The Cretaceous flora, Pt. 1. *London*.

STUART, CAPT. MURRAY.—(1923) The geology of Takki Zam valley, and the Kaniguram-Makin area, Waziristan. *Rec. Geol. Surv. Ind.*, Vol. LIV, No. 54.

Explanation of Plate VI

All figures are of natural size unless otherwise stated. The figured specimens are preserved in the Geological Survey of India, Calcutta.

Fig. 1. *Algites* cf. *A. intricatus* (Sternb.). Thallus showing irregularly dichotomous and pinnate branching. The specimens are chiefly confined to the right hand half of the photograph.

(a) *Algites* cf. *A. Targionii* (Brongn.). Thallus slightly broader.

(b) *Algites* sp. Thallus unbranched, made up of small bladder-like structures (cf. Fig. 5 b which is an enlarged photograph of the specimen shown in Fig. 3 b).

Fig. 2. *Algites* cf. *A. intricatus* (Sternb.).

(a) *Algites* cf. *A. Targionii* (Brongn.).

Fig. 3. *Algites* cf. *A. intricatus* (Sternb.).

(b) *Algites* sp. Same species as the one shown in Fig. 1 b. The specimen is shown enlarged in Fig. 5 b.

Fig. 4. *Algites* cf. *A. intricatus* (Sternb.). Thallus cylindrical possibly due to the mode of preservation. The cylindrical appearance is not clearly seen in the photograph.

(b) *Algites* sp. Thallus cylindrical possibly due to the mode of preservation; probably the same species as those shown in Figs. 1 b, 3 b, 5 b.

Fig. 5. *Algites* sp. Same specimen seen in Fig. 3 b enlarged to show irregular outlines of thallus formed of bladder-like structures. ×Ca 4¼.

Fig. 6. *Algites* cf. *A. Meyrati* (Fisch.-Oost.). Thallus irregularly branched, branches broad and of unequal length.
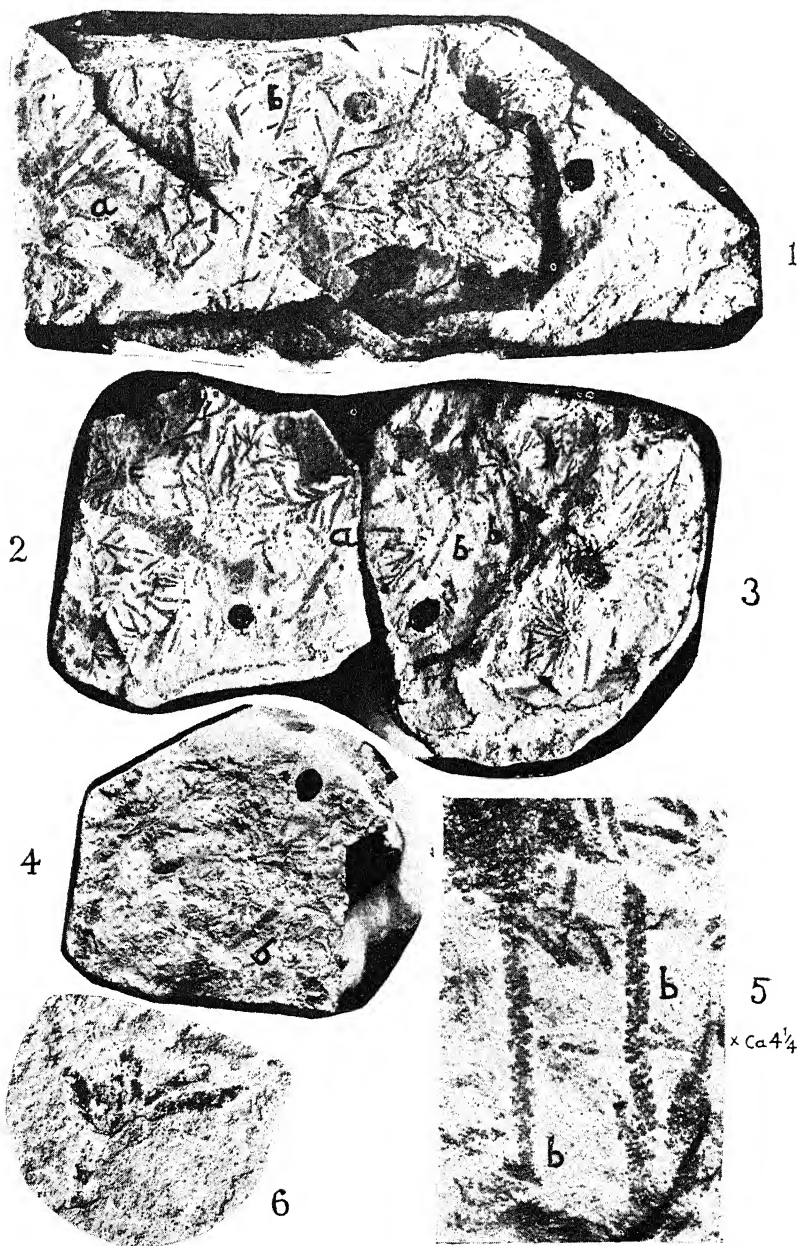
FUNGI OF NAINITAL

The fungi enumerated in "Fungus Flora of Nainital" Part I, (Jour. Ind. Bot. Soc. Vol. XI, No. 2, pp. 178—180, 1932.) were collected only during the month of October while those given in this second list of Nainital fungi have had a wider seasonal range, being collected off and on during May, June, July, September and October.

Fifteen of the species and one genus are new to Science : they are marked with a double asterisk. Another fifteen species, marked with a single asterisk, are on new hosts. Nineteen fungi, marked with a X, are not mentioned by Butler and Bisby in "The Fungi of India."

No.	Name of fungus.	Name of host.
	Phycomycetes.	
*1.	Cystopus bliti (Biv.) de Bary.	Achyranthes aspera var. porphyristachya.
	Ascomycetes.	
**2.	Phyllachora Tandonii Mitter.	Ficus foveolata.
*3.	Phyllactinia corylea (Pers.) Karst.	Jasminum sp.
4.	Schizothyrium annuliforme Syd. & Butl.	Acer sp.
	Basidiomycetes.	
	Uredinales.	
**5.	Aecidium callianthum Syd.	Desmodium tiliaefolium.
*6.	Aecidium cunninghamianum Barclay.	Cotoneaster microphylla.
7.	Aecidium flavescens Barclay.	Senecia rufinervis.
**8.	Aecidium leucadinum Mitter.	Leucas mollissima.
**9.	Aecidium strobilanthinum Mitter.	Strobilanthes alatus.
**10.	Aecidium Tandonii Mitter.	Deutzia staminca.
11.	Chnoopsora Sancti—Johannis (Barcl.) Diet.	Hypericum cernuum.
12.	Coleosporium campanula (Pers.) Lev.	Campanula colorata.
13.	Colcosporium clematidis Barclay.	Clematis montana.
14.	Coleosporium leptodermidis (Barclay.) Syd.	Leptodermis lanceolata.

No.	Name of fungus.	Name of host.
**15.	*Coleosporium Mitteri* Syd.	*Barleria cristata.*
**16.	*Coleosporium myriactidis* Syd.	*Myriactis nepalensis.*
17.	*Coleosporium perillæ* Syd.	*Perilla ocimoides.*
*18.	*Coleosporium plectranthi* Barclay.	*Plectranthus Cœtsa.*
X 19.	*Coleosporium senecionis* Fr.	*Senecio sp.*
20.	*Melampsora yoshinagai,* P. Henn.	*Wikstrœmia canescens.*
21.	*Monosporidium andrachnis* Barclay.	*Andrachne cordifolia.*
22.	*Phakopsora cronartiiformis* (Barclay) Diet.	*Vitis himalayana.*
23.	*Phragmidium rosæ-moschatæ* Diet.	*Rosa moschata.*
*24.	*Phragmidium sp.*	*Potentilla lcchenanthana.*
**25.	*Puccinia adjuncta* Mitter.	*Artemesia parviflora.*
X 26.	*Puccinia bullata* (Pers.) Went.	*Pimpinella acuminata.*
X 27.	*Puccinia bullata* (Pers.) Went.	*Selinum tennifolium.*
28.	*Puccinia bupleuri-falcati* (DC.) Went.	*Bupleurum falcatum var. marginata.*
29.	*Puccinia caracis-filicinæ* Barclay.	*Carex filicina.*
*30.	*Puccinia circææ* Pers.	*Circæa lutitiana.*
**31.	*Puccinia cutela* Syd.	*Thalictrum punduanum.*
*32.	*Puccinia ferruginosa* Syd.	*Artemesia Roxburghiana.*
33.	*Puccinia iridis* (DC.) Wallr.	*Iris.*

No.	Name of fungus.	Name of host.
*34.	*Puccinia leucophaea* Syd. & Butl.	*Colquhounia vestita.*
35.	*Puccinia persistens* Plowr.	*Thalictrum foliolosum.*
36.	*Puccinia polliniæ* Barclay.	*Strobilanthes Dalhousianus.*
X 37.	*Puccinia polygoni* Alb. et Schw.	*Polygonum alatum* var. *nepalensis.*
X 38.	*Puccinia punctata* Lk.	*Galium asperifolium.*
X 39.	*Puccinia punctata* Lk.	*Galium Mollugo.*
40.	*Puccinia saxifragæ-ciliatæ* Barclay.	*Saxifraga ligulata.*
41.	*Puccinia taraxaci* (Rebent.) Plowr.	*Taraxacum officinale.*
42.	*Puccinia urticæ* Barclay.	*Urtica parviflora.*
43.	*Puccinia violæ* (Schum.) Dc.	*Viola serpens.*
44.	*Pucciniastrum coriariæ* Diet.	*Coriaria nepalensis.*
X 45.	*Ravenelia japonica* Diet. & Syd.	*Albissia odoratissima.*
**46.	*Ravenelia Mitteri* Syd.	*Indigofera gerardiana.*
*47.	*Ravenelia Mitteri* Syd.	*Indigofera leptostachya.*
*48.	*Ravenelia Mitteri* Syd.	*Indigofera pulchella.*
*49.	*Ravenelia Mitteri* Syd.	*Indigofera sp.*
**50.	*Uredo verecunda* Syd.	*Achyranthes bidentata.*
X 51.	*Uromyces capitatus* Syd.	*Desmodium tiliaefolium.*
**52.	*Uromyces clivalis* Mitter.	*Argyrolobium flaccidum.*

No.	Name of fungus.	Name of host.
*53.	Uromyces strobilanthis Barclay.	Strobilanthes alatus.
54.	Uromyces Superfluus Syd.	Panicum antidotale.
55.	Uromyces Trigonellœ Pass.	Trigonella Emodi.
X 56.	Uromyces Valeriana-wallichii (Diet) Arth. et Cumn.	Valeriana Wallichii.
	(c) **Hymenomycetes**	
57.	Coprinus sp.	
X 58.	Fomes caryophylli (Rac.) Bres.	
59.	Hydnum sp.	
60.	Polyporus calcuttensis Bose.	
X 61.	Polyporus gilvus-forma gilvoides Schw.	
62.	Polyporus Hookeri Llyod.	
X 63.	Polyporus similis.	
X 64.	Polystictus cuticularis.	
65.	Sterium sp.	
	Fungi Imperfecti	
**66.	Aulographopsis indica Petrak (New genus).	Pteris sp.
**67.	Chatomella indica Syd.	Coriaria nepalensis.
X 68.	Coniosporium Bambusae (Theum et Bolle) Sacc.	Bamboo packing.

7

No.	Name of fungus.	Name of host.
X 69.	Melasmia Lonicerae Jacz.	Lonicera quenquelocularis.
X 70.	Phomopsis calystegiae (cke) Petr.	Ipomae sp.
*71.	Phyllosticta ambrosioides Theum.	Chenopodium giganteum.
**72.	Sarcinella Tandonii Mitter.	Euonymus tingens.
73.	Septoria cannabis (Lasch.) Sacc.	Cannabis sativa.
X 74.	Septoria cerastii Rob.	Cerastium vulgatum.
*75.	Septoria hyalina Ell et Ev.	Viola serpens.
**76.	Septoria Mitteriana Syd.	Lysimachia chenopodioides.
*77.	Septoria violae West.	Viola serpens.
X 78.	Spilosticta geranii (Fr.) Pet.	Geranium Wallichianum.
X 79	Stigmina platini (Fuck) Sacc.	Platanus orientalis.

SOME OBSERVATIONS ON RIGHT- AND LEFT-HANDED ASYMMETRY IN SOUTH INDIAN AROIDS

BY

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In 1932 in making observations on several species of *Arisaema* in the Nilgiris (Journ. Bomb. Nat. Hist. Soc. Vol. XXXVII, No. 3), it was noticed that in some plants the right side of the spathe of the inflorescence overlapped the left side, while in other specimens of the same species the reverse was the case. This overlapping of one side of the spathe by the other is accompanied by a spiral twisting of the upper end of the spathe in the bud, some spathes being twisted so as to resemble a right-handed screw and others so as to resemble a left-handed screw; in some cases the edges of the cataphylls and leaf blades or bases also overlap in the same direction as the spathe. As in some genera of Monocotyledons, such as *Dioscorea,* the direction of twining is sufficiently definite and constant for this character to be used as a key-factor for the species, it was thought to be of interest to find whether the species of other genera of the family *Araceae* resemble those of *Arisaema* in not showing a constant direction of twisting and overlap.

Below are recorded the place of growth and the number of individuals with right-handed and the number with left-handed spathes for all plants of the various species of *Araceae* that have come under the writer's observation during the past few years. The identity of each species has been confirmed at Kew either by Mr. C. E. C. Fischer or by the writer.

		Left	Right
(1) *Cryptocoryne retrospiralis* Kunth			
Karapara River, Cochin	..	27	14
Tenmalai, Travancore	..	74	91
Mettur, Salem District	..	19	15
		120	120

			Left	Right
(2)	*Cryptocoryne consobrina* Schott			
	Karapara River, Cochin	..	21	21
(3)	*Cryptocoryne spiralis* Fisch.			
	Lonavla, Bhor Ghat	..	13	14
(4)	*Lagenandra ovata* Thw.			
	Tenmalai, Travancore	..	13	38
	Kottayam, Travancore	..	3	2
			16	40
(5)	*Lagenandra toxixaria* Dalz.			
	Tenmalai, Travancore	..	17	16
(6)	*Lagenandra sp.*			
	Nilgiri Wynaad	..	2	3
(7)	*Acorus Calamus* Linn.			
	Kandale Valley, Travancore	..	7	4
	Ootacamund Lake, Nilgiris	..	3	2
			10	6
(8)	*Typhonium trilobatum* Schott			
	Tubers from West Coast, grown at			
	Tambaram		2	5
(9)	*Typhonium flagelliforme* Bl.			
	Tubers from West Coast, grown at			
	Tambaram		7	20
(10)	*Theriophonum minutum* Engl.			
	Tambaram and District (1933-36)..		114	94
(11)	*Theriophonum Wightii* Schott.			
	Tambaram, Chingleput District	..	1	1
(12)	*Theriophonum infaustum* N. E. Br.			
	Tenmalai, Travancore	..	1	1
	Chalakudi, Cochin	..	3	5
	Grown at Tambaram	..	8	6
			12	12

		Left	Right
(13)	*Ariopsis peltata* Nimmo		
	Munnar, Travancore	6	10
(14)	*Colocasia antiquorum* Schott		
	Chalakudi, Cochin	18	14
(15)	*Xanthosoma cordifolium* N.E.Br.		
	Cultivated and run wild in Madras	7	4
(16)	*Remusatia vivipara* Schott		
	Nilgiri Wynaad	3	1
(17)	*Arisaema attenuatum* Bar. & Fisch.		
	Munnar, Travancore 1935	22	36
	,, ,, 1937	38	44
		60	80
(18)	*A. Barnesii* Fisch.		
	Longwood Shola, Nilgiris	29	27
	Neduvattam, Nilgiris	13	18
	Ouchterlony Valley, Nilgiris	24	20
		66	65
(19)	*A. Leschenaultii* Bl.		
	Ootacamund Downs, Nilgiris	84	90
	Longwood Shola, Nilgiris	52	66
	Travancore High Range	12	10
		148	166
(20)	*A. peltatum* Fisch.		
	Munnar, Travancore	13	20
(21)	*A. psittacus* Bar.		
	Mannavan Shola, Travancore	13	22
(22)	*A. sarracenioides* Bar. and Fisch.		
	Munnar, Travancore 1935	30	18
	,, ,, 1937	19	24
		49	42

		Left	Right
(23) *A. tortuosum* Schott			
Neduvattam, Nilgiris	..	11	9
Banagudi Shola, Nilgiris	..	7	22
		18	31
A. tortuosum var. *neglectum* Fisch.			
Mercara, Coorg	..	1	3
A. tortuosum non-typical form with 5 leaflets.			
Dodabetta, Nilgiris	..	6	6
(24) *A. translucens* Fisch.			
Thia Shola, Nilgiris	..	30	23
(25) *A. tuberculatum* Fisch.			
Pennant Shola, Nilgiris 1933	..	18	15
,, ,, ,, 1935	..	8	9
		26	24
(26) *A. tylophorum* Fisch.			
Thia Shola and Neduvattam, Nilgiris			
1933	..	25	36
Neduvattam 1935	..	77	105
		102	141
(27) *A. Wightii* Schott			
Anamudi, Travancore 1935	..	14	8
,, ,, 1937	..	30	27
Dodabetta, Nilgiris	..	2	7
		46	42
(28) *Amorphophallus sylvaticus* Kunth			
Tambaram, Chingleput District			
1934	..	26	36
,, ,, 1935	..	34	35
,, ,, 1937	..	12	6
		72	77

		Left	Right
(29) *Anaphyllum Wightii* Schott			
Kavali, Cochin	..	1	2
Ponmudi, Travancore	..	1	1
		2	3
(30) *Richardia africana* Kunth			
Ootacamund Lake, Nilgiris	..	14	18
Palaar, Travancore	..	3	7
		17	25

In *Pistia stratiotes,* Linn. and *Pothos scandens,* Linn. the spathe is not twisted and one side does not overlap the other, at least in the mature inflorescence.

These observations appear to lead to the following conclusions :—

(1) In none of the species studied is the asymmetry exclusively right- or left-handed. As the plants observed belong to thirteen widely varying genera, it appears probable that this is also true of the family *Araceae* as a whole.

(2) In most cases (Nos. 1, 2, 3, 5, 12, 14, 18, 25, 27 and 28) there are the same or nearly the same number of individuals showing right-handed asymmetry as those showing left-handed asymmetry. This suggests that in these cases the variation is due to chance.

(3) Omitting the cases where the number of specimens counted is obviously too small to justify any numerical conclusions being drawn, it is seen that with a number of the remaining species (Nos. 4, 17, 23 and 26 and possibly 10 and 19) the departure from equality is greater than would be expected from random variation. There appear to be two possible explanations of these inequalities depending on the mode of reproduction of the plant. Most of these aroids produce seeds and also reproduce vegetatively by bulbils, underground runners or creeping rootstocks. As is indicated below, there is some evidence that in a number of species it is not merely the spathe that is either right-handed or left-handed, but the whole plant has a right- or left-handed bias. If this be the case, plants produced vegetatively would generally be expected to show the same asymmetry as the parent. In a restricted area, such as a single shola or valley, most plants are likely to have had a common origin, and if reproduction were chiefly vegetative, a local predominance of right- or left-handed individuals would not be surprising. In making the counts it was observed with some of the *Arisaemas* and with *Acorus* that the plants were often found in groups and that in some cases the spathes of all the plants in a group were similar in their asymmetry. Probably these groups were produced vegetatively

from the same parent. The observed inequality in such cases would thus appear to be due to the limited area over which the observations were made, and it is to be expected that if a sufficiently large number of plants from the whole area of distribution of a species were counted, the numbers of the two kinds would approximate to equality.* On the other hand, if asymmetry were a Mendelian character and if in any particular species right- or left-handedness were a dominant, then reproduction by seed would also result in unequal numbers of individuals showing the two kinds of asymmetry. No evidence from breeding experiments with these species has so far been obtained in support of this possibility, and the numerical proportions found in the cases where there was not approximate equality do not appear to approach the ratio to which this assumption would lead.

Although a species as a whole is not exclusively right- or left-handed, it is of interest to find whether an individual plant is constant in this respect. Information on this subject is not very easy to obtain by observation in the field, as in many species only one inflorescence is produced in a season and often only one after several seasons. The only species in which more than one spathe produced by the same plant have been seen are (3) *Cryptocoryne spiralis* (5) *Lagenandra toxicaria* (8) *Typhonium trilobatum* (9) *Typhonium flagelliforme* (12) *Theriophonum infaustum and* (13) *Ariopsis peltata.* (3) occasionally bears two or three inflorescences at the same time in different axils. One plant had three spathes all right one had two both right, one had two both left. One plant, however, had one right-handed and one left-handed spathe. (5) has a horizontal succulent stem and several inflorescences are produced at intervals during a season at different nodes. In several marked plants, the two or three spathes produced were similar in asymmetry. In (8) the blade of the leaf is rolled up in the bud so that the inner side is completely wrapped round by the outer; sometimes it is the right and sometimes the left side that is outermost. A number of inflorescences are produced each season. One plant while under observation produced four leaves and three inflorescences all right; another, one leaf and three inflorescences all left; another, two leaves and one inflorescence all left. One plant, however, produced one right and one left leaf, and two left and one right inflorescences. In this species the two sides of the blade of the leaf are not symmetrical, the side which was outermost in the bud being more deeply lobed at the base than the other. This lack of symmetry

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*It may be of interest to record that in 1934, with a view to testing whether by counting a very large number of spathes the result would be approximate equality, a count was made of as many spathes as possible of the common English aroid *Arum maculatum*. It was found that of 982, 508 were left and 474 were right. In this case also there was some evidence of local predominance, but apparently the numbers counted and the area covered were insufficient to settle the question being tested. *Acorus Calamus* is the only aroid·that is found wild·both in England and in S. India. Specimens counted in England were found to be 27 right and 12 left.

is most distinct in young leaves but it often persists in the fully developed leaf. With (9) one plant produced two inflorescences and five leaves, all right, and three other plants produced several leaves and inflorescences of similar asymmetry. One plant of (12) produced three inflorescences all left and another two inflorescences both right. In the only plant of (13) seen to produce two inflorescences, the spathes overlapped in opposite directions.

Some observations have also been made with cataphylls and leaf bases. In *Arisaema Leschenaultii* there are several membranous cataphylls clasping the base of the petiole. In the early stages of growth, one side of the cataphyll overlaps the other. Of 56 plants observed in the Nilgiris, 49 were found to have a cataphyll and the spathe overlapping in the same direction, and in 7 cases they were opposed. A specimen of *A. Leschenaultii* from Coorg had 5 cataphylls and the spathe all with the left side over the right. In *Arisaema*, the cataphylls are homologous with the petioles of the leaves, and a minute vestigial leaf blade with several lobes can sometimes be seen at the tip. In *A. Leschenaultii,* with rare exceptions, only one leaf is produced each season. The lower part of the petiole of this leaf clasps the base of the peduncle. The petiole is cylindrical and succulent, and externally there is little to indicate any overlapping. If, however, the upper part of the plant is cut off near the base, it is usually seen that one side of the petiole is overlapping the other, although the sides up to a certain height are joined internally by a thin membrane. In 17 specimens in which this overlapping was quite clear, 16 had the overlapping of the petiole base and the spathe in the same direction and one in the opposite direction. In the non-typical form of *A. tortuosum* with 5 leaflets there are usually two leaves. Of 13 plants examined, 6 had the spathe and both leaf bases overlapping in the same direction, 4 had 2 the same and the other missing or not overlapping; and in only one case did the same plant have one leaf base right and the other left. The only specimen of *A. sarracenioides* examined had the spathe, a cataphyll and the base of the petiole all left over right. A specimen of *A. Wightii* from Dodabetta had 2 cataphylls, 2 leaf bases and the spathe all right, but another had a cataphyll, a leaf base and the spathe right but the other leaf base left. In *Cryptocoryne retrospiralis* numerous leaves are present on a plant at the same time. By cutting across the plant near the bases of the leaves, it is seen that the petiole bases overlap in a cyclic manner. Plants in which the leaf bases overlapped to the left were found to bear spathes twisted as a left-handed screw, and those whose leaf bases overlapped to the right had spathes with a right-handed twist.

It thus appears that in at least some species of aroids, each individual plant has a right- or a left-handed tendency although in a certain proportion of individuals this tendency appears to be weak or absent.

In most species referred to above the asymmetry consists of a simple overlapping of one side of the spathe by the other, often accompanied by a

twisting of the upper part of the spathe; but there are a number of cases in which the asymmetry is different, and the following notes are added in explanation.

Cryptocoryne and *Lagenandra* differ from the other genera in that the tube of the spathe is modified to a chamber which opens to the limb by a valve, the opening of the valve being to one side of the chamber and the spadix to the other. In (1) and (2) there is a long twisted cylinder above the chamber, and it is difficult to say by inspection which is the front of the spathe and, consequently, whether the valve or the spadix is on the right; above the cylinder, however, there is a twisted limb; it is the direction of twist of the limb that is recorded. In (3), (4), (5) and (6) there is no cylindrical portion above the chamber and the limb is closed at first but opens by a slit, the lower part of which is arc-shaped. By comparing the elements of asymmetry present in the various species of these two genera, it is found that the following go together or are equivalent: (a) right side of spathe overlapping left in mature spathe or in bud (b) spathe twisted so as to resemble a right-handed screw when mature or in bud (c) valve to right of spadix (d) arc-shaped lower end of opening of spathe concave to the right. Plants having one or more of these elements are called "Right" in the above record, and their opposites or mirror images are recorded as "Left". *Acorus Calamus* is quite different from all the others. In this species the spadix is attached about half way up what appears to be a long ensiform leaf. The part of this apparent leaf below the spadix is the modified peduncle and the part above is the modified spathe. The peduncle is wedge-shaped in cross-section and there is a groove along the blunt edge. The ridge on one side of this groove is wider than the ridge on the other, and in the spathe this wider ridge becomes the mid-rib while the narrower ridge continues as one of the edges of the spathe. The spathe is thus asymmetrical, and in some specimens the broader ridge that becomes the mid-rib is on the right and in others on the left. Further, the peduncle is twisted through about a right angle between top and bottom. It is found that a right-handed twist of the peduncle goes with a broad ridge on the right, and these appear to correspond with a right-over-left overlap in other genera.

Summary

Observations on the right- and left-handed asymmetry of the spathes of 30 species of aroids found in S. India have been made. In most species there are found to be about equal numbers of plants having right- and left-handed spathes and it appears probable that observations on larger numbers would show this to be true of the others also. Some evidence has been obtained that each individual plant has a right- or left-handed bias, but there appear to be exceptions.

ON THE STRUCTURE AND LIFE-HISTORY OF
PSEUDOVALONIA FORBESII (Harv.) IYENGAR
(*VALONIA FORBESII* Harv. *)

## (Preliminary note)

BY

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Living material of this alga was brought from Pamban and grown at Madras in the laboratory and the several stages of its life-history were studied in some detail. This alga was until now placed in the genus *Valonia*, but a study of its structure and development shows that it is not a *Valonia*. The peculiar lenticular cells and the tiny marginal cells bearing short rhizoids which are so characteristic of *Valonia* are never formed in this alga. And, unlike *Valonia*, but more like *Ernodesmis, Siphonocladus, Struvia* and *Chamaedoris,* the base of the alga forms an annularly constricted stipe. (Text-figs. 2 and 4). Boergesen first drew attention to this peculiarity of *Valonia Forbesii* in his recent paper, " Some Marine Algae from Ceylon " (Ceylon Journ. Sc., Vol. XII, pt. 2, 1936, p. 62, fig. 1). The plant is attached to the substratum by horizontal creeping rhizoidal branches formed from the basal portion of the stalk. And these rhizoidal branches, through segregative cell division, soon become septate. Some of the cells of these septate rhizoids swell up and grow into secondary vesicles around the parent vesicle (Text-figs. 2—4), so that the fully grown plant appears like a cluster of vesicles (Text-fig. 1). This method of proliferation from the septate rhizoidal portion is quite similar to what is seen in *Chamaedoris Peniculum* (Sol.) O. Kuntze as recorded by Boergesen (Marine Algæ of the Danish West Indies, Vol. I, p. 57, fig. 40), but quite unlike what is seen in *Valonia utricularis* (Roth) Ag. as recorded by Kuckuck (Ueber den Bau und die Fortpflanzung von *Halycystis* Aresch. und *Valonia* Ginn. Bot. Zeit. 1907). A new genus *Pseudovalonia* is therefore created for this alga. (See also " A note on two interesting South Indian

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* Alg. Ceylon, No. 75 (Nomen nudum). (Quoted from Boergesen below). J. G. Agardh: Till Algernes Systematik VIII, p. 96 F. Boergesen, Some Marine Algæ from Ceylon, Ceylon Journ. Sci. (A) Vol. XII, Pt. 2, 1936, p. 62, fig. 1.

Marine Algæ, *Pseudovalonia Forbesii* (Harv.) Iyengar comb.
nov. and *Pseudobryopsis pambanensis* sp. nov.: Proceedings of
the Twenty-fifth Indian Science Congress, Calcutta, 1938,
pp. 132 and 133, Pt. III, Abstracts).''
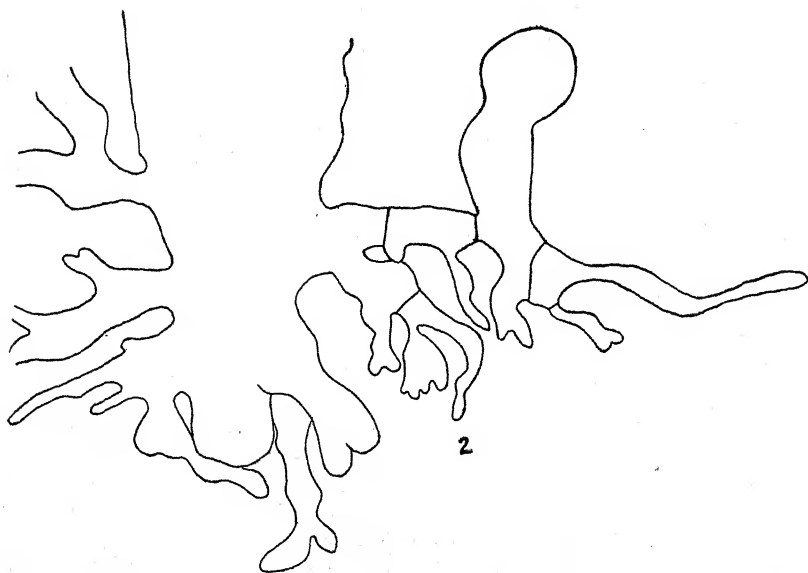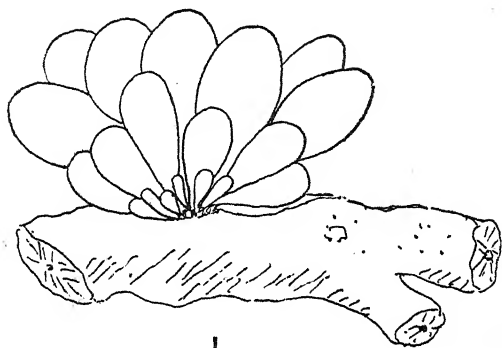
Text-figs. 1-2.—*Pseudovalonia Forbesii.*   Fig. 1,   General clustered
habit of the plant.   About natural size.   Fig. 2. Base of the plant showing
rhizoids formed from the stalk; some of the rhizoids have become septate
and young vesicles are already formed from some of the cells. × about 40,
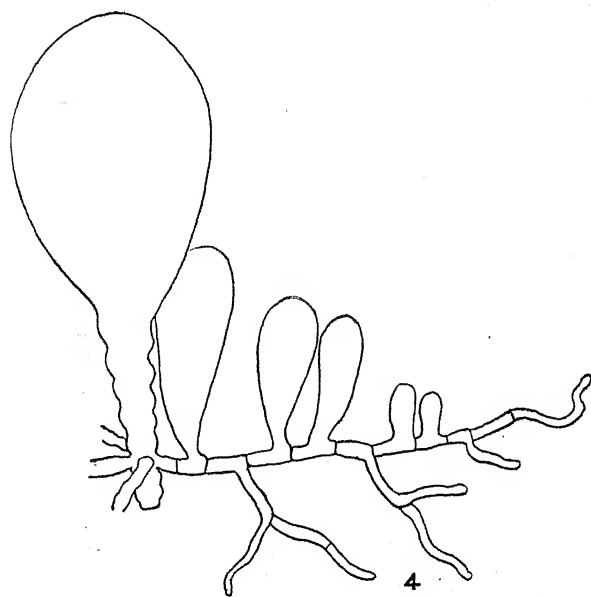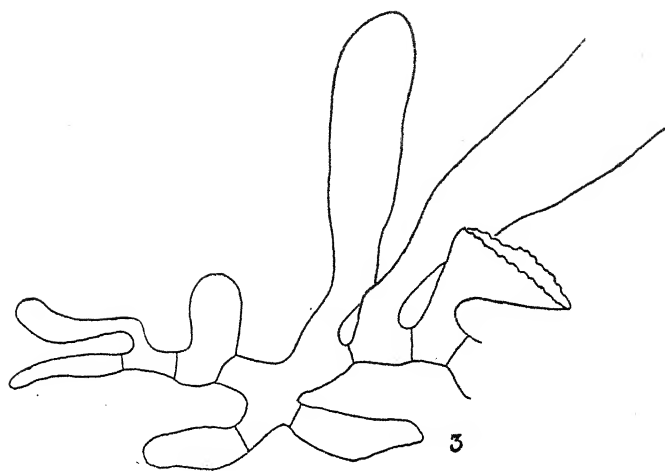
Text-figs. 3-4.—Fig. 3. A proliferating rhizoidal portion with the vesicles grown larger. × about 40. Fig. 4. A diagrammatic representation of the structure of the alga, showing the annular stalk and the proliferation of secondary vesicles from a septate rhizoid.

Under unfavourable conditions, plenty of cysts are formed inside the vesicle. These cysts grow into new plants when conditions become more favourable again.

Numerous four-ciliated motile spores are formed in each vesicle. These escape outside through a large number of round apertures formed in the wall of the vesicle and, after swarming for some time, finally settle down and grow into young plants. No case of sexual fusion was observed. The occurrence of four-ciliated swarmers in this alga is interesting, since usually only biciliated swarmers are formed in the *Valoniaceae*. The only previous record of four-ciliated swarmers is by Kuckuck *(loc. cit.)* in *Valonia macrophysa* Kutz.

Description

Pseudovalonia gen. nov.

Thallus vesicular and narrowed downwards into an annularly constricted stalk ; thallus attached to the substratum by rhizoids formed from the base of the stalk : rhizoids soon becoming septate through segregative cell-division : plenty of new vesicles proliferating all round the base of the parent vesicle through the enlargement and growth upwards of the cells of the rhizoidal portion, giving the alga a clustered appearance ; lenticular cells never formed in the thallus.

Asexual reproduction through the formation of numerous four-ciliated swarm spores formed inside the vesicle.

Sexual reproduction not known.

RESPIRATION OF AMPHIBIOUS PLANTS
I. SCIRPUS ARTICULATUS LINN.

BY

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An amphibious plant has two phases to cover its life-cycle. The early stage is under water and the later stage is aerial or *vice versa*. The same plant lives quite efficiently in both the phases. As respiration affords a convenient key to understand the life-activities of an organism, the respiration of an amphibious plant throughout its life-cycle was considered worth investigation. Moreover people have studied the respiration of aerial plants (7) as well as of water plants (4), but there has been no account of respiration of an amphibious plant in both the phases of its life-cycle. Of course, there are accounts of aerial organs as to their respiration under submerged condition (7), but that only gives some idea of respiration under abnormal conditions. This study aims at giving an account of the respiration of the plant both under submerged and aerial conditions which happen to be normal environments to the plant during some stage of its life or other.

Material

The plant chosen for the study was *Scirpus articulatus* Linn. an amphibious plant belonging to the family Cyperaceae. They grow abundantly near water edges. There is a marked peculiarity in the plant that the leaves are linear borne on a condensed conical stem which gives rise to a tuft of roots. The plant remains in this form for nearly a half of its life-cycle, being submerged under water. The leaves never come up to air. Later on leaves begin to die out and small cylindrical hollow shoots known as scapes develop. These scapes contain air and when the plant is matured they become completely aerial, the leaves in the meanwhile are replaced by scale leaves and the whole plant is nothing but a number of scapes. Flowers are borne on the nodal regions of the scapes.

For the study of respiration a large number of plants of the same age, each in a pot, were kept submerged. The work was started when the plants were fifteen days old and continued through the ontogeny of the plant.

Method

As the plant has two phases in its life-cycle, *viz.*, (i) the submerged phase and (ii) the aerial phase, the respiration was studied by two different methods. For the submerged phase, the method of knowing respiration by the change of pH value of the surrounding water as described by Smith (6) was followed and for the aerial stage the usual method of absorbing CO_2 by Baryta was adopted.

1. *The pH method.*—Here the plants were taken out of water, thoroughly washed in tap water and kept in it for two hours to allow the plants to get adapted to the new conditions. The pH value of the tap water was determined by the use of Phenol red as indicator. Ten gms. of plant material was then put in fifty c.c. of tap water (the pH of it being known) in a resistant glass tube with the indicator (Phenol red) in the proportion of three drops per 10 c.c. of water and then the tube was corked. The pH value of the tap water used was 7·6. When the pH value of the water in the tube changed to 6·6, which could be determined by comparison with a buffer standard, the plants were taken out of that water, washed thoroughly and treated as before. Each time, the time taken for the change of pH value due to respiratory CO_2 was noted. By this method a relative idea of the respiration under water from day to day could be obtained. From these values the absolute quantities of respiratory CO_2 were calculated after knowing the amount of CO_2 that was responsible for the change of pH value of the tap water from 7·6 to 6·6 by the two following methods:—

(*a*) Fifty c.c. of tap water of 7·6 pH with the proportionate amount of indicator was taken and definite quantities of CO_2 were allowed to be absorbed in the water till the required pH of 6·6 was obtained. The weight of CO_2 that gave the required change was calculated to be 1·53 mgs.

No. of Expt.	CO_2 in mgs. for the definite pH change in 50 c.c. of water.	Mean
1	1·53	
2	1·54	
3	1·52	1·53
4	1·53	
5	1·53	

(*b*) Secondly, in each of two tubes 50 c.c. of tap water was taken and in one the proportionate amount of indicator (3 drops of 0·01% solution per 10 c.c.) was added and equal quantities by weight of plant material were kept in both the tubes to respire. When the definite change to pH 6·6 was reached in the tube containing the indicator the plants were taken out. To the water of the tube, without the indicator, excess of N/150 Ba (OH)$_2$ was added to absorb the respired CO_2 together with the CO_2 that was dissolved before. The excess of Baryta was titrated against a standard solution of N/200 HCl. From this the dissolved amount of CO_2 was determined. Again, the dissolved CO_2 in the tap water was determined by absorbing it by N/150 Baryta, the excess of which was titrated against N/200 HCl. From the difference of the two values, the amount of CO_2 responsible for the change of pH from 7·6 to 6·6 was obtained. It was found that the value thus obtained was the same (1·53 mgs.) as that obtained by absorption method.

The respiratory values, as has been stated, were recorded in time, during which the definite change of pH value occurred. From these values, the amount of CO_2 respired by 10 gms. of plant material per hour, every day, was calculated. Thus it was possible to obtain an absolute value for respiration under water.

2. *The Baryta method.*—Here the value of respiratory CO_2 was obtained by absorbing the CO_2 in Baryta in petten-koffer tubes and titrating the excess of Baryta by standard HCl. When the plants became aerial the respiration was obtained by this method.

The values of submerged respiration and aerial respiration showed a very striking difference and to throw light on this, the respiration of scapes in water containing various amounts of O_2 was studied. The value of O_2 content of water was increased by passing O_2 from a cylinder, the amount of O_2 in water being determined by Winkler's method (8).

The respiration of scapes, free from air (*i.e.*, injected with water) was also studied to find out any relationship of air content of the scapes with the respiration intensity. For similar reasons also, the rate of growth of the scapes was observed from day to day.

The experiments were carried on under constant temperature, *viz.*, between 30 degrees centigrade and 31 degrees centigrade. The growth rate was recorded when the plant was growing in its natural surroundings.

9

Results

The results are represented graphically and in tabular forms.

TABLE 1

Respiration of the plants under water for 41 days since the 15th day of their age.

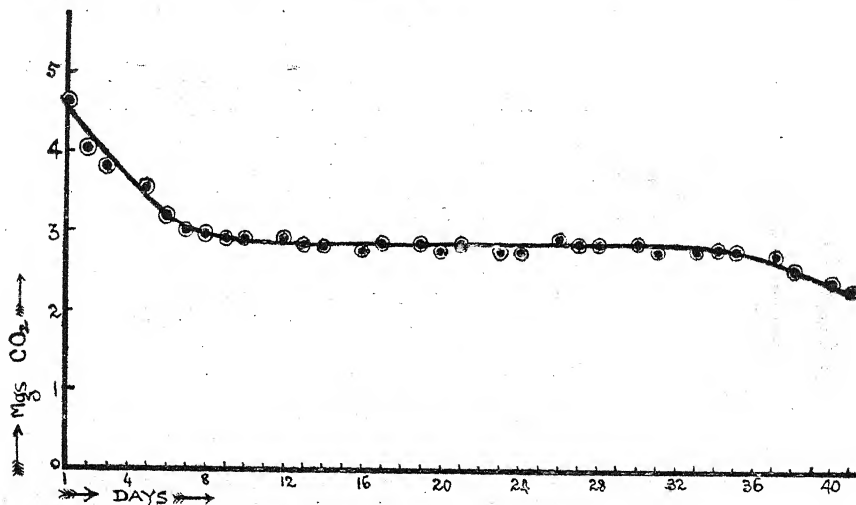| Days	Mgs. of CO_2 per hour per 10 gms. of plant material.	Days	Mgs. of CO_2 per hour per 10 gms. of plant material.
1	4·602	22	..
2	4·054	23	2·789
3	3·835	24	2·789
4	..	25	..
5	3·567	26	2·931
6	3·196	27	2·876
7	3·068	28	2·856
8	2·988	29	..
9	2·876	30	2·789
10	2·876	31	2·789
11	..	32	..
12	2·876	33	2·789
13	2·848	34	2·823
14	2·823	35	2·789
15	..	36	..
16	2·789	37	2·707
17	2·876	38	2·556
18	..	39	..
19	2·876	40	2·422
20	2·789	41	2·301
21	2·853	..	..

Fig. 1. Showing respiration of the submerged plants, beginning from the 15th day of their age.

TABLE 2

Respiration of submerged scapes beginning from the time when they were 3 days old.

Days	Mgs. of CO_2 per hour per 10 gms. material.	Days	Mgs. of CO_2 per hour per 10 gms. material.
1	3·068	9	4·844
2	3·285	10	4·602
3	..	11	4·602
4	..	12	4·468
5	4·002	13	4·428
6	4·72	14	..
7	..	15	4·403
8	5·113	..	..
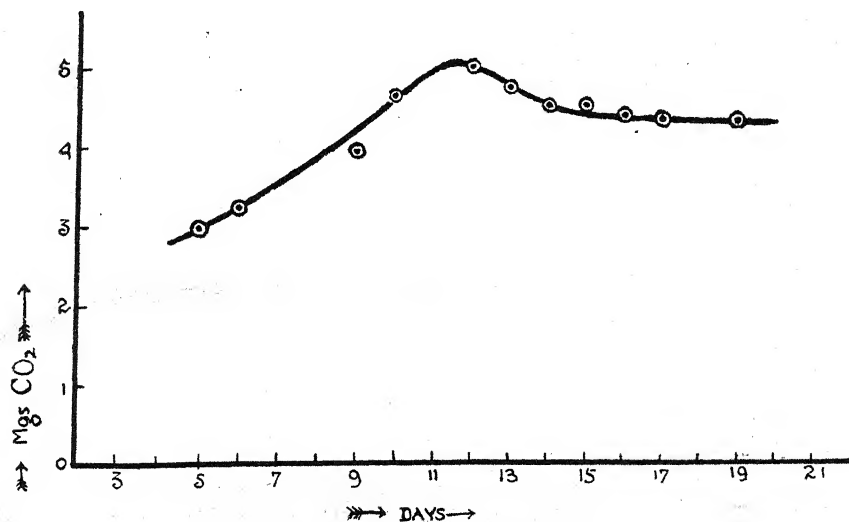
Fig. 2. Showing respiration of the submerged scapes, since the 3rd day of their age.

TABLE 3

Respiration of aerial scapes.

Days.	Mgs. of CO_2 per hour per 10 gms. material.	Days.	Mgs. of CO_2 per hour per 10 gms. material.
1	5·32	24	5·82
2	5·39	25	6·23
3	5·36	26	6·44
4	5·322	27	..
5	5·43	28	..
6	..	29	..
7	5·325	30	6·63
8	5·33	31	6·66
9	5·42	32	6·653
10	..	33	6·64
11	5·412	34	..
12	5·396	35	6·582
13	..	36	6·54
14	5·397	37	6·2
15	5·402	38	5·97
16	5·441	39	5·662
17	..	40	5·43
18	5·389	41	..
19	..	42	5·21
20	5·42	43	4·73
21	5·49	44	4·31
22	5·51	45	3·85
23	5·7	..	..
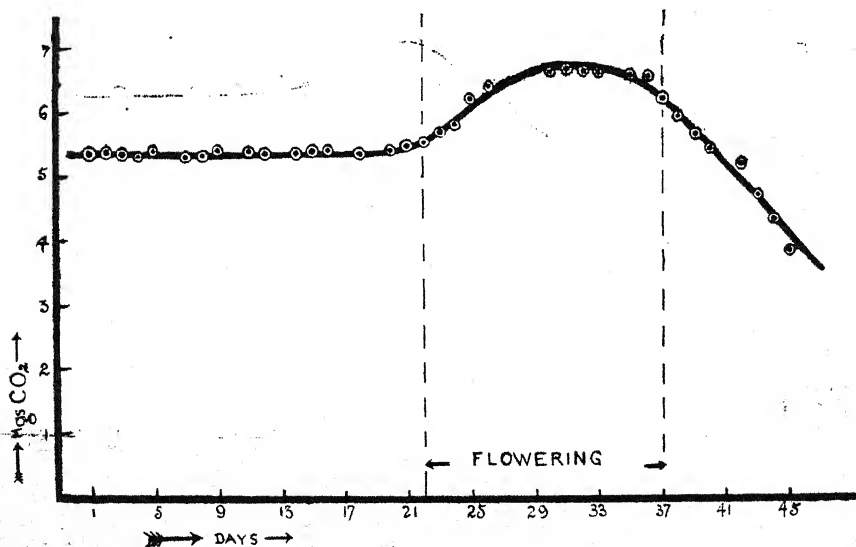
Fig. 3. Showing respiration of the aerial scapes.

TABLE 4

Respiration of scapes in various percentages of O₂ in water, given in mgs. of CO₂ per hour per 10 gms. material.

Boiled tap water *i.e.,* in 0% O₂	Tap water *i.e.,* 0·56% O₂	Water containing 1·56% O₂	Water containing 2·41% O₂	Aerial respiration.
2·51	3·99	4·6	4·909	5·346
2·51	3·99	4·55	5·14	5·354
2·57	4·1	4·65	4·909	5·352
2·51	3·99	4·65	4·909	5·348
2·46	4·1	4·55	5·139	
Mean 2·512	4·03	4·6	5·0	5·35
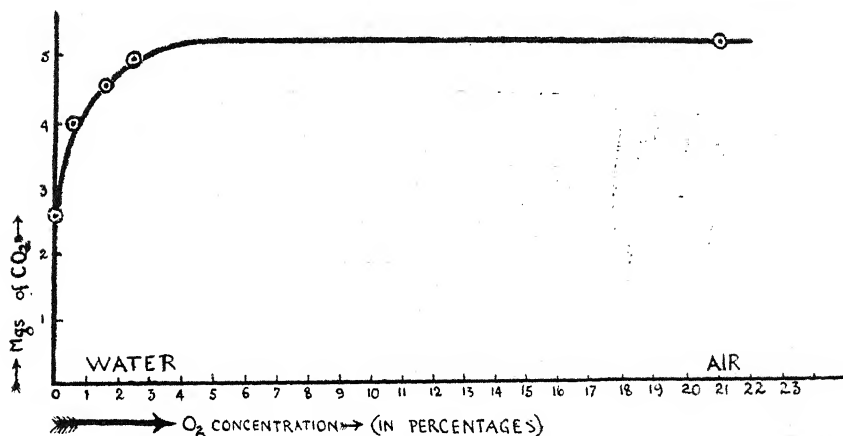
Fig. 4. Showing respiration of the scapes in different concentrations of O₂.

TABLE 5

Respiration of air free scapes in various percentages of O₂ given in mgs. of CO₂ per hour per 10 gms. material.

Boiled tap water 0% O₂	Tap water 0·56% O₂	Oxygenated water 1·56% O₂
2·65	3·72	4·38
2·66	3·8	4·29
2·64	3·68	4·325
2·7	3·77	4·32
2·69	3·75	4·35
2·52	3·73	4·329
Mean 2·643	3·79	4·33

TABLE 6

Rate of growth of the scapes, beginning from the 3rd day of their age.

Days	Rate of growth per two days in cms.
1	0·5
3	1·7
5	2·7
8	5·2
10	3·6
12	2·9
14	1·8
16	0·9
18	0·4
20	0·4
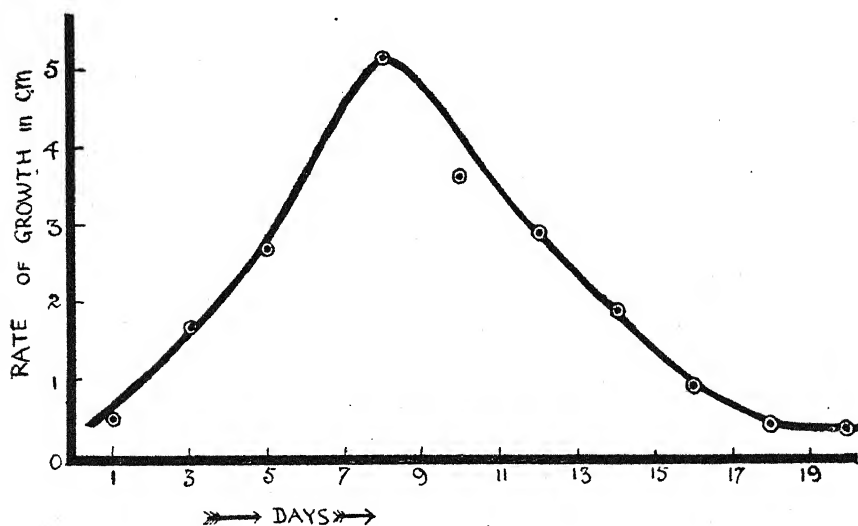
Fig. 5. Showing rate of growth in length of the scapes since they were three days old.

From the graphs and the tables it is clear that the plant under submerged conditions has high respiration at the early stage and then comes to the level phase, during which the march of respiration remains steady for a pretty long time and after that the respiration falls off. A similar fate is to be met with in the case of scapes respiring under water—a rise with level phase. During the aerial life of the scapes, the level phase which is attained after the submerged stage, continues for a longer time, when again, there happens a gradual rise in respiration at the flowering period of the scapes. After this the respiration falls off. But a peculiarity that one notices in the respiratory curve of the aerial scapes is this that the rate of respiration in its level phase is greater than that under submerged conditions, *i.e.*, the level phase attained in air is higher than that obtained under water. This is all about the difference between the respiration in both its phases of life-cycle of an amphibious plant.

Now, when we turn to the respiratory values of the scapes in water containing various percentages of O_2, the experiments that were chiefly undertaken to clarify, if possible, the differences as shown above, it is seen that the respiratory value goes on increasing with the increase of oxygen concentration of the water, till the concentration of oxygen reaches 5%. Same thing happens in the case of air free scapes, though the respiratory value is less than that in normal scapes, *i.e.*, scapes containing air in intercellular spaces.

Again, looking at the growth curve, we find that during the early period the growth rate is greater, *i.e.*, the rate rises quickly and then descends and keeps up a uniform rate after a period of 20 days.

Discussion

One interesting point that emerges from the investigations is that the respiration under water is limited by O_2 supply. As the absorption coefficient of O_2 in water is very low, *i.e.*, 2·6% at 30° C. and 2·3% at 40° C., the O_2 available to the plant is very meagre. So, it can be said that the respiration that is taking place under water is mostly anaerobic. In the case of *Scirpus articulatus*, we find increased respiratory activity with the increase of O_2 concentration in the water in which the plant respires. In our case the scapes that had low respiratory values under water had a higher respiratory value when they became aerial and in that case the high value of respiration can thus satisfactorily be ascribed to the increase of O_2 in the surroundings of the plant, *i.e.*, air.

Besides this the high value of respiration during the early periods may be ascribed to growth. Again, the respiration of the submerged scapes is greater than that of submerged plants before the scapes are formed. This also is due to greater O_2 content of the hollow scapes. In our experiments it is seen that air free scapes (scapes injected with water) have lesser respiration than normal

scapes. This, surely, supports our opinion as to the higher respiration of the submerged scapes than that of the submerged plants.

Therefore, lastly, in concluding, we can say that the difference that we come across in the respiratory value of the plant in the submerged and aerial stages is due to oxygen concentration. In a case of submerged phase oxygen concentration limits the respiratory activity, which rises in air due to increased oxygen concentration.

In the end, I take this opportunity to tender my thanks to Professor P. Parija, M.A. (Cantab), I.E.S., for his keen interest and valuable suggestions in the work.

Summary

1. The material and the methods of finding respiration are described.

2. Respiration during the submerged phase is determined by the pH method.

3. Respiration of submerged scapes is recorded by the same pH method.

4. The aerial respiration of the scapes found out.

5. Respiration of scapes under water in various percentages of O_2 investigated.

6. It is found that the aerial respiratory values of the scapes are greater than those of the submerged phase.

7. This difference is ascribed to the low concentration of dissolved oxygen in water.

References

1. BARTON-WRIGHT (1934).—Recent advances in Plant Physiology.

2. CAVEN, R. M. (1921).—Foundations of Chemical Theory, page 148.

3. OSTERHOUT (1918).—Journ. Gen. Physiol., Vol. 1, No. 17.

4. PAL, N. L. (1934).—Studies in the Respiration of Conjugating Spirogyra with special reference to fat metabolism. New Phytol, Vol., 33, No. 4, page 241.

5. SMALL, JAMES (1929).—Hydrogen ion concentration in plant cells and tissues.

6. SMITH, E. P. (1924).—Effect of general anaesthetics on the respiration of Cereals. Annals of Botany, Vol. 38 page 261.

7. STILES and LEACH (1932).—Respiration in Plants.

8. TREADWELL (1930).—Analytical Chemistry, Vol. 2, page 654.

CONTRIBUTIONS TO A SOUTH INDIAN MARINE ALGAL FLORA.—III.

BY

F. Boergesen

The Botanical Museum of the University, Copenhagen

Communicated by M. O. P. Iyengar

In this the third and last part of these contributions are enumerated the remaining species of Professor Iyengar's collections which I have been able to determine. Owing to the lack of sufficient material, there being often only a single specimen of each species, the determinations have often been difficult and somewhat uncertain, especially in the case of critical and polymorphic forms of which a large number of specimens are necessary in order to give an account of the variations. I wish to thank Professor Iyengar once more most heartily for his kindness in entrusting me with the work of determining his collection, as it is always very interesting to be able to work on the flora of a more or less unknown area. Professor Iyengar has taken very great interest in my algological researches in South-India. He met me at Karvar and later on during my stay at Tuticorin very kindly arranged for boats and a good working place also there. But for his kind help, I am afraid the result would have been very meagre.

Besides IYENGAR's collection and my own from Tuticorin, I have received from the British Museum (Nat. Hist.), London, a collection of South–Indian algae for determination. The main part of this consisted of specimens collected by Professor IYENGAR and presented by him to the British Museum. Included in this collection was a small one by EDGAR THURSTON most of the specimens of which were from the Madras beach and a few from Tuticorin.

Finally Professor W. RANDOLPH TAYLOR, Ann Arbor, Michigan, has been kind enough to send me a small collection of South Indian algae for determination. Most of these specimens were collected near Trivandrum, Travancore, and a few were from Krusadi Island, Pamban.

Of the Phæophyceæ and the Rhodophyceæ, (as mentioned already in the previous parts, Professor IYENGAR wishes to investigate the Chlorophyceæ himself), I found in all 38 species of the former and 114 species of the latter, but after investigations of new material from South Indian shores the number is sure to be highly increased. The species of *Sargassum* are not included in the present list. Professor IYENGAR's collection of this genus has been determined by Professor W. A. SETCHELL and is now at Madras.

Even if it is of course a little too early to say anything about the geographical position of the South-Indian algal flora, I wish to point out that several species, as might be expected, are also found in the flora of Ceylon as well as in the flora of the Indian-Malayan Archipelago. On the other hand many of the species found in the northern part of the Arabian Sea are not met with in the southern part of India.

DR. H. E. PETERSEN has most kindly determined the specimens of *Ceramium* which I have come across in the material. I wish to thank him most sincerely for doing this for me.

As I intend to stop, at any rate temporarily, my investigations of Indian algæ, I wish to thank all those who kindly sent me material of Indian algae and other material for purposes of comparison and who have helped me in other ways, for all their kindness.

I wish to add that the habit illustrations in this part have for the most part been drawn by the young artist, stud. mag. H. HÖVRING and a few by MISS INGEBORG FREDERIKSEN.

Copenhagen in January 1938.

PHÆOPHYCEÆ

I. Ectocarpales

Fam. 1. **Ectocarpaceæ**

Hecatonema Sauvag.

1. **Hecatonema sargassicola** nov. spec.

Frons plana, ca. 2 mm. alta, maculas plus minus expansas, irregulares in foliis *Sargassi* format. Stratum basale unistratum compositum ex filamentis horizontalibus irregulariter ramosis et sinuosis, inter se conjunctis, articulatis, articulis, diametro (6-7μ longo) plus minus longioribus. Fila erecta dense aggregata, brevia aut longa, ad 2 mm. longa et 6-7μ crassa, sporangia seriata aut sparsa aut terminalia gerentia. Sporangia pluriloculariasessilia aut pedicellata, oblonge ovata, ca. 10-13μ longa et 8-11μ lata.

I n d i a: Cape Comorin, Oct. 1924, leg. M. O. P. I.

This plant forms quite low, soft and dense expansions upto about 2 mm. high on the leaves of a *Sargassum*. The tufts may cover nearly the whole leaf of the host plant and are very irregular in shape and become most probably enlarged owing to the growing together of several small tufts.

The base (Fig. 1a) consists of creeping filaments, which near the edge are more or less free but later coherent. They are composed of irregular roundish-oval or elongated cells about 6-7μ broad and upto 15μ long and 7μ high. From the cells in this disc the erect filaments grow up. Most of the filaments become long, but some remain short, being composed of only a single or a few cells, the uppermost of these cells being transformed into a plurilocular sporangium (Fig. 1a). The long assimilating filaments reach a length of up to about 2 mm. and are 6-7μ thick, and consist of cells 10-20μ long. The cells contain one or more parietal irregularly shaped chromatophores. These were very much damaged as they had been kept for a long time in formalin. Up along the assimilating filaments, often from near their base, but generally in the middle, plurilocular sporangia are developed. The sporangia are sometimes sessile (Fig. 1d), but most often pedicellate, the stalk being composed of a single or two to three cells (Fig. 1b, c). They are as a rule placed unilaterally up along the filaments, a sporangium being developed from nearly every cell in the filaments (Fig. 1b). The sporangia, however, are also often irregularly alternating or scattered (Fig. 1c). Only plurilocular sporangia are found. They are oblong-oval in

shape and about 10-13μ long and 8-11μ broad. When a sporangium
is emptied, a new one is often developed from the base of the old
one, and as this process may take place several times, the walls
of the previous sporangia are often found surrounding the last
sporangium (Fig. 1a, c). The terminal cells in the long assi-
milating filaments are often transformed into a sporangium
(Fig. 1f). Once near the apex of a filament, I found a cell
which was transformed into a sporangium (Fig. 1g), but this I
am sure is quite an exceptional case.
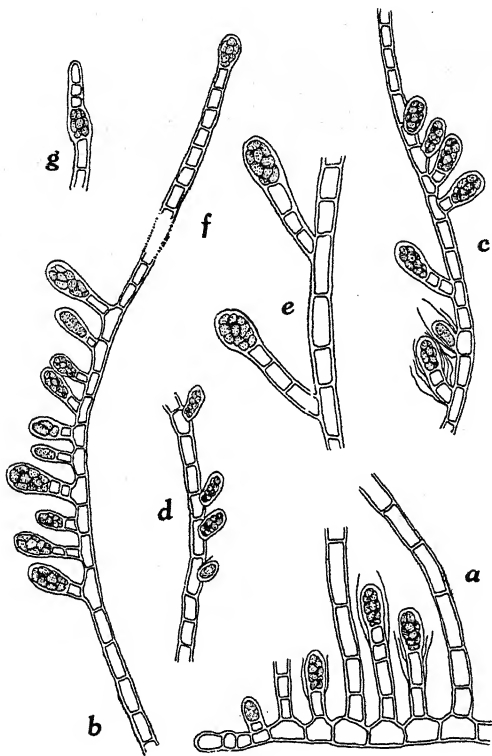
Fig. 1.—*Hecatonema sargassicola* nov. spec. *a*, base of a piece of a
plant in transverse section; *b, c, d, e*, parts of filaments with sessile or
pedicellate plurilocular sporangia; *f*, terminally placed sporangium; *g*, an
intercalary sporangium, *a, e*, × 600; *b, c, d, f, g*, × 450.

As said above, the sporangia may sometimes have a short
stipe composed of a few cells, but real branches growing out to
long filaments like the mother filaments were not observed by me.
I have been looking in vain for hairs. These are evidently
completely wanting.

This species seems to be rather closely related to a plant
which in The Marine Algae of the Faeröes (Botany of the

Faeröes, part II, Copenhagen 1902, p. 424, fig.   79) I have called *Myrionema faeroense*, but which quite recently in agreement with our present definition of these forms by Levring in his valuable paper, Zur Kenntnis der Algenflora der norwegischen Westküste, p.   47, is referred to the genus *Hecatonema*.   It is, however, easy to separate the Faeröese plant from the Indian one owing to its much longer, elliptical-cylindrical, plurilocular sporangia.   The Indian plant has no hairs and neither did I find any in the Faeröese material examined by me, but Levring found hairs in the specimens from the West coast of Norway referred by him to *Hecatonema faeröense*.

II.   Sphacelariales

Fam. 1. ***Sphacelariaceæ***

Sphacelaria Lyngb.

1.   Sphacelaria tribuloides Menegh., Lett. a Corinaldi, p. 2, n. 1 (after De-Toni, Syll. Alg. vol. III, p. 502).   Sauvageau, Remarques sur les Sphacélariacées, p. 123, figs. 28-29 (Journal de Botanique, vol. XV, 1901).

On an old battered and undeterminable stem of a fucaceous plant cast ashore on the beach at Madras some small tufts of this species were present.   The plant had propagula and a few unilocular sporangia. *Sphacelaria furcigera* Kütz. mentioned in Part I, p. 359 of these Contributions was found intermingled in the tufts.

India: Madras Beach, Febr. 1900, coll. by Edgar Thurston no. 73 (in the British Museum, Nat. Hist., London).

D i s t r.: Most warm and temperate seas.

RHODOPHYCEÆ

I.   Nemalionales

Fam. 1. ***Chætangiaceæ***

Scinaia Bivona.

1.   Scinaia bengalica Boergs., Two species of Scinaia from South-India (Botaniska Notiser, Lund 1938, p. 89).

I n d i a: Madras Beach, Febr. 1900, collected by K. Rangachary.

2.   Scinaia carnosa Harv. cfr. Boergesen, l. c. p. 94.

I n d i a: Cape Comorin, Sept. 1900, leg. M.O.P.I.

D i s t r.: Ceylon, South India.

II. Gelidiales

Fam. 1. **Gelidiaceæ**

Gelidiella Feldm. et Hamel.

1. Gelidiella Bornetii (Web. v. B.) Feldm. et Hamel in Revue Géner. de Bot., t. 46, 1934, p. 528.—*Gelidium Bornetii* Web. v. B., Algues de 1, expedition danoise aux iles Key (Vidensk. Medd. Dansk naturh. Foren., vol. 81, Koebenhavn 1926, p. 107).

A small *Gelidiella* (Fig. 2) creeping on a piece of *Lithothamnion* seems to agree quite well with Mme. WEBER's description. The decumbent creeping filaments are fixed to the substratum by means of vigorous rhizoids. From the basal part the erect filaments arise reaching a height of about 3-5 mm. The filaments are about 100-200μ broad and in the fruiting parts up to 250μ or more. In tranverse sections, they are more or less oval, showing that the thallus is somewhat compressed. They become more so in the upper fruiting parts. In the lower parts of the filaments the cortical cells are quadrangular, higher up they are roundish and somewhat smaller. They are rather distinctly seriately arranged.

While Mme. WEBER's specimens were sterile, the specimens examined by me had tetrasporangia. These occur crowded together in roundish-oblong clumps in the upper somewhat broadened and flattened ends of the erect filaments. The diameter of the tetrasporangia is about 27μ. In a later paper (Rèsultats Scientif. du Voyage aux Indes orient. Néerlandaises, etc. publiés par V. VAN STRAELEN, vol. VI, fasc. 1, Algues. Bruxelles 1932, p. 20, pl. III, fig. 5-6) Mme. WEBER points out that the thallus traverses the body of the corals. The specimen seen by me was as mentioned above growing on a piece of *Lithothamnion*, and, because of the scarcity of the material I had for examination, I was not able to see if the plant was able to penetrate the thallus of the alga.

In its size, manner of growth and general habit, this plant reminds one of *Gelidiella tenuissima* Feldm. et Hamel, Gélidiales (Revue Algologiques, t. IX, 1936, p. 226, figs. 11-12), but the Indian plant is easy to recognize owing to the different arrangement of its tetrasporangia, these being seriately arranged in *Gelidiella tenuissima*.

I n d i a : Krusadi Island, Sept. 25, leg. M. O. P. I. (no. 55).

D i s t r.: Kei Islands.

2. Gelidiella myrioclada (Boergs.) Feldm. et Hamel, l. c., p. 529.—*Echinocáulon myriocladum* Boergs. in Kew Bull., 1934, p. 5, figs. 4, 5.

A few small sterile specimens fixed to shells and stones were dredged at a depth of about 10 meters. The specimens were not much ramified. The diameter of the thallus was about 100μ.

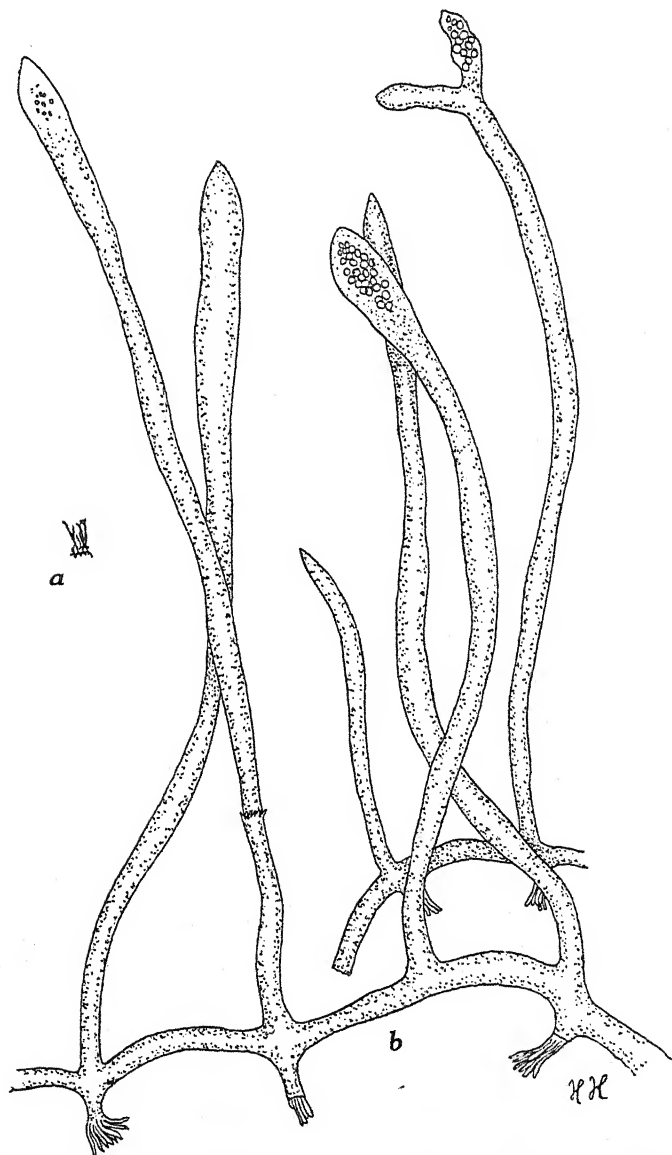I n d i a : In the sea off Tuticorin, March 1928 (!).
D i s t r.: Bombay.

Fig. 2.—*Gelidiella Bornetii* (Web.) Feldm. et Ham. *a*, tuft of the plant (natural size) ; *b*, about. ✕ 20.

3. Gelidiella acerosa (Forssk.) Feldm. et Hamel, Observations sur quelques Gélidiacées in Revue Gén. de Bot., t. 46, 1934, p. 528. BOERGESEN, Mar. Algae from Ceylon, p. 80.—*Fucus acerosus* Forssk., Fl. Ægypt.-Arab. (1775) p. 190. *Echinocaulon acerosum* (Forssk.) Boergs., Revision Forssk. Algae p. 5. *Fucus rigidus* Vahl, Skrivter of Naturh.-Selskabet vol. 5, Kiöbenhavn 1802, p. 46. *Gelidium rigidum* Grev. in Montagne, Algues de Cuba, p. 45.

I n d i a : Krusadi Island, May, 1924, leg. M. O. P. I.

D i s t r.: Most warm seas.

Gelidiopsis Schmitz.

1. Gelidiopsis variabilis (Grev.) Schmitz.

To this species mentioned in part II of these contributions I believe a slender form collected by me on the shore of the Hare Island near Tuticorin is to be referred. The tufts reach a height of 3-4 cm. and the filaments vary in breadth from about 500μ in the thicker ones to about 100μ in the thin ones. In List mar. Alg. Bombay, p. 44, I have mentioned a similar small form.

I n d i a : Tuticorin, Hare Island, March 1928 (!).

D i s t r.: Indian Ocean.

Gelidium Lamour.

1. Gelidium micropterum Kütz., Tab. Phycol., vol. 18, p. 21, pl. 59. Cfr. *Gelidium corneum* (Huds.) Lamour. in Contrib. to a South Indian marine algal flora, I, p. 46.

There has been for quite a long time considerable doubt as to which plant exactly the name *Fucus corneus* Huds. (*Gelidium corneum*) refers. Owing to this uncertainty, DE-TONI in Sylloge gave up altogether employing this name. In 1931 SETCHELL* undertook a very thorough examination and after much investigation arrived at the conclusion that a specimen of BUDDLE's in Herb. SLOANE at the British Museum should be the type specimen of *Fucus corneus*; but alas, this specimen was a form of *Gelidium sesquipedale* Thuret and did not belong to any of the forms (especially of *Gelidium pulchellum* and *G. latifolium*) which have been considered to be *Gelidium corneum*. Because of his discovery SETCHELL is of opinion that *G. sesquipedale* Thuret ought to be called by HUDSON's name. Against this the French algologists FELDMANN and HAMEL, who have contributed so much to our knowledge of the *Gelidiales*, have protested in a recent paper,

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* SETCHELL, W. A. Some early algal Confusions (University of Calif. Publicat. in Botany, vol. 16, 1931, p. 358).

Floridées de France. Gélidiales, in Revue Algologique, t. IX, p. 230. They point out that taking up for *Gelidium sesquipedale* the very ambiguous name *corneum* as proposed by Setchell will be a source of constant mistakes and confusion, and they think it would be best to let the name sink into oblivion for ever.

I refer the reader to Feldmann and Hamel's papers as well as to that of Setchell, so that I need not go into details regarding this question, but I wish to point out that, in agreement with the above mentioned authors, it seems better to give up using the specific name "*corneum*", at any rate for the plant, which in the first part of these Contributions, p. 46, and in List mar. Alg. Bombay, p. 43, and in Some Marine Algae from Ceylon, p. 80, has been called by the specific name *corneum* and instead to take up Kützing's name *Gelidium micropterum* with the figure of which the specimens from India and Ceylon agree very well.

I n d i a : Cape Comorin, Sept. 1924, leg. M.O.P.I.

D i s t r .: Cape, India.

III. Cryptonemiales.

Fam. 1. *Corallinaceæ*.

Amphiroa Lamour.

1. Amphiroa fragilissima (L.) Lamour., Hist. Polyp. Corallig. Flexib. p. 298. Areschough in J. Agardh, Spec. Alg., p. 531. Weber van Bosse, and M. Foslie. The Corallinaceæ of the Siboga-Exp. p. 89, pl. XVI, figs. 1, 2, 5. — *Corallina fragilissima* L., Systema Nat., ed. 12, I, p. 1305.

I n d i a : Pamban, Oct. 1922, leg. M.O.P.I.

D i s t r .: Much distributed in warm seas.

Cheilosporum Aresch.

1. Cheilosporum spectabile Harv., Friendly Island Algæ no 31 (nomen nudum). Grunow, Alg. Fidschi, etc. p. 41. Weber van Bosse and Foslie The Corallinaceæ of the Siboga-Exp., p. 106.

Some specimens found at Karvar agree very well with the form found by me at Bombay; compare the figure 23 in "List mar. Alg. Bombay", p. 51—52. The specimens were sterile.

I n d i a : Karvar, Febr. 1928, leg. M.O.P.I., (!).

D i s t r .: Malayan Archipelago, Bombay, Polynesia.

Jania Lamour.

1. Jania rubens (L.) Lamour., Hist. Polyp. Corallig. Flexib., p. 272. ARESCHOUGH in J. AGARDH, Spec. Alg., II, p. 557. KÜTZING, Tab. Phycol., vol. VIII, tab. 84.

A few specimens in IYENGAR's collection seem referable to this species; the thallus is about 120-150μ thick.

I n d i a : Cape Comorin, Sept. 1924, and Krusadi Island, "on coral stones," leg. M.O.P.I.

D i s t r.: Seems to be much distributed in warm seas.

Fam. 2. *Grateloupiaceæ*

Halymenia Ag.

1. Halymenia dilatata Zan. in Flora, 1851, p. 35; Plant. mar. Rubr. enum. p. 72. tab. III, fig. 1. J. AGARDH, Analecta Algologica, 1892, p. 53. Okamura, Icones, vol. IV, p. 109, pl. 176 et 177, figs. 3-4.

From Malvan I have seen a dried female specimen which agrees very well with ZANARDINI's description and figures with the exception of the thallus not being maculated. A transverse section of the thallus shows that, under ·the rather thin cortical layer composed of small roundish or oblong cells, thin irregularly bent filaments run in all directions into the slimy interior. But I have not seen any authentic specimen for comparing with it.

The specimen was collected and most kindly sent to me by Mr. S. C. DIXIT, BOMBAY.

I wish to add that in the collection of the British Museum (Nat. Hist.) a single small specimen is present which agrees fairly well with ZANARDINI's figure in having a maculated thallus, but the structure of the thallus is different from that of the above-mentioned specimen from Malvan, since its cortical cells are much larger, the filaments in the interior are few, and, when soaked in water, the transverse sections are slow to assume their natural size. This specimen is tetrasporic, the tetrasporangia being cruciately divided. It was collected at the Madras Beach, Febr. 1900. Coll. EDGAR THURSTON (no. 76). If the first mentioned specimen is correctly named, thus belonging to *Halymenia dilata*, the latter is surely another species, but, in order to decide this, more and better preserved material is necessary.

D i s t r.: Red Sea, Somaliland, Malayan Archipelago, Japan.

Grateloupia C. Ag.

1. Grateloupia filicina (Wulf.) Ag., compare Boergesen, Mar. Alg. Bombay, p. 53, where literature is quoted.

Specimens with tetrasporangia were gathered in February, 1928.

I n d i a : Tuticorin, M. O. P. I.

D i s t r .: Most warm seas.

2. Grateloupia lithophila nov. spec.

Frondes cæspitosæ, planæ, undulatæ et sinuosæ, lanceolate-lineares, ad 12 cm. altæ et ca. $\frac{1}{4}$-1 cm. latæ, a media parte utrinque attenuatæ, simplices aut irregulariter divisæ, marginibus nudis, aut in superiore parte thalli, ubi thallus sæpe truncatus est prolificationes gerentia.

Substantia, ut videtur, gelatinose-membranacea, specimina exsiccata chartæ arctius adhærent. Color brunneo-olivaceus in violaceum transiens.

Tetrasporangia cruciatim divisa per totam frondem sparsa.

I n d i a : Madras, Jan. 1926, leg. M.O.P.I. no. 370 (type); ibid. Febr. 1900 leg. K. Rangachary (specimens in Herb. Iyengar and in the British Museum (Nat. Hist.).

The plant (Plate VII) grows in tufts upon stones near the shore and reaches a height of about 12 cm.; the breadth of the thallus varies very much from about $\frac{1}{4}$-1 cm. The thallus is simple or divided, linear-lanceolate, tapering from the middle towards both ends; it is flat and more or less sinuate and undulating. The upper ends of the fronds are often truncate becoming more or less broadly rounded, and from the upper ends of these fronds proliferations are usually given out; otherwise the margins are as a rule bare.

The colour of the dried specimens are mostly dark olive-brown with an occasional tinge of violet. The plant adheres strongly to paper and when alive is sure to have been slippery. Only tetrasporic specimens are present; the tetrasporangia are cruciately divided and scattered over the surface of the plant.

As to the shape of the thallus this species seems to have an intermediate position between *Grateloupia prolongata* J. Ag. and forms of *Grateloupia cuneifolia* J. Ag. with narrow leaves. De-Toni in Sylloge IV, p. 1565, mentions that *Grateloupia prolongata*, according to Piccone, was found at Colombo, Ceylon. The plant referred to by Piccone is most probably the same as the one described here. This species seems also to resemble *Gr. furcata* Holmes, but, according to the description, Holmes's plant, of which I have seen no specimens, is rather regularly dichotomously divided and does not taper towards the apex and, if so, only very little.
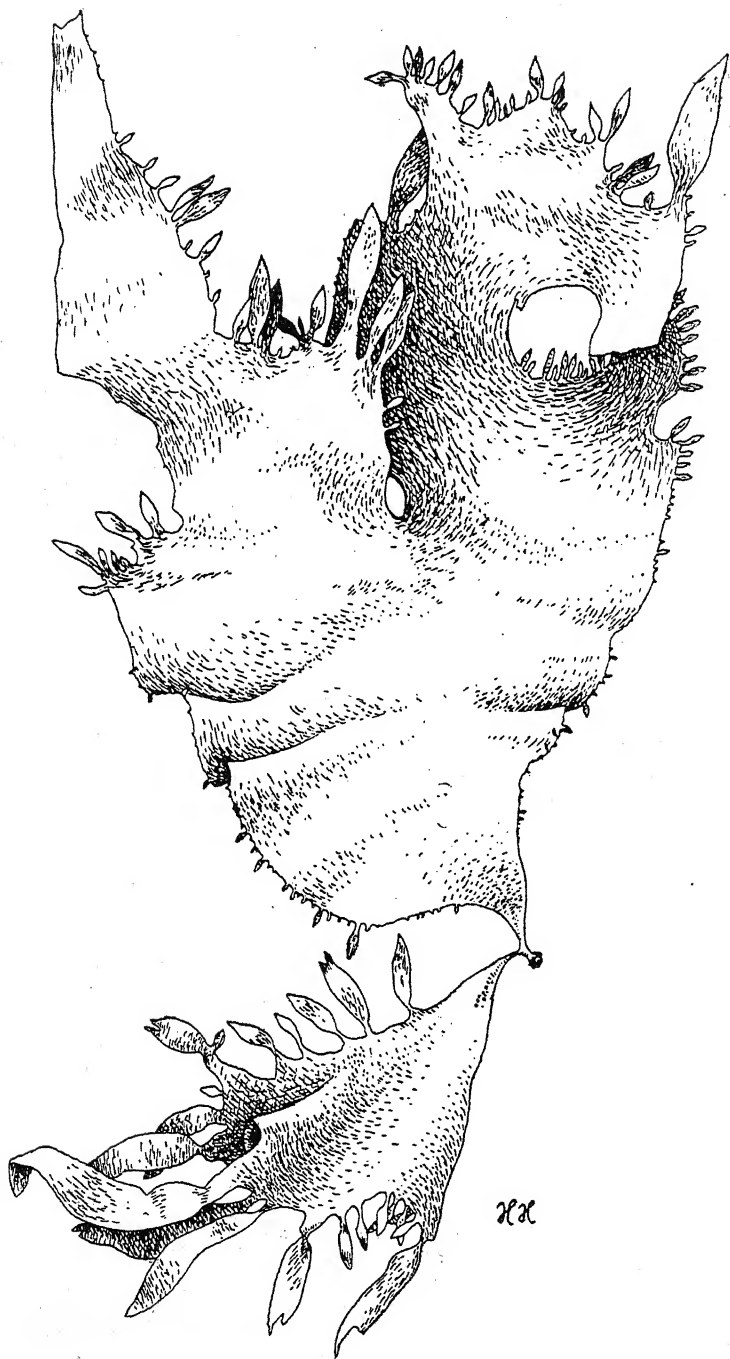
Fig. 3.—*Grateloupia Comorinii* Boergs.—About 2/3 natural size.

3. Grateloupia Comorinii nov. spec.

Frondes usque ad 30 cm. altæ, gelatinose-carnosæ, membranaceæ, a disco parvo basali solitariæ aut gregatim proveniunt, simplices aut plus minus divisæ, denique sæpe valde irregulariter formatæ.

Frons basi late cuneata, nunc oblonge-lanceolata, nunc late-obovata, superne sæpe plus minus truncata, marginibus sinuosis et undulatis, integerrimis aut hic illic prolificationes numerosas gerentibus. Color brunneo-violaceus.

Tetrasporangia cruciatim divisa per totam frondem dispersa.

I n d i a : Cape Comorin, Sept. 1924. leg. M.O.P.I. no 274 (type).

From a small basal disc a very short stripe arises which immediately is broadened out making an even transition into the leaf-like frond; in some cases a few (2-4) fronds issue from the disc or from the lowermost part of the stipe. The base of the frond is cuneate, but otherwise the shape of the plant (of which I have seen altogether 7 specimens) varies very much (Figs. 3 and 4). In some of the specimens it is elongated-obovoid and gradually tapering upwards, in others rather abruptly truncate, which is due probably to some sort of damage. The upper margins in these truncate specimens are more or less deeply sinuate or laciniate (Fig. 3). In one specimen the frond was rather deeply divided into two halves. The margins of the thallus are more or less sinuate and undulating. While some of the specimens are quite or almost completely destitute of proliferations having an entire and even margin (Fig. 4), others have a great number of smaller or larger proliferations developed generally somewhat above the base. These proliferations are, however, most numerous and vigorous along the upper margins of the truncate specimens (Fig. 3).

To illustrate the size of the plant, I wish to give here the measurements of some of these. A small specimen (Fig. 4a) with an oblong elongated frond tapering gradually upwards and with nearly entire margins was 14 cm. long and $5\frac{1}{4}$ cm. at its broadest, which is $4\frac{1}{2}$ cm. above the stipe. Another larger specimen (Fig. 4b) with an oblong-linear frond and rather undulated margins and a few rather large proliferations was 31 cm. long and about 6 cm. broad. The specimens with truncate ends were proportionately much broader; thus the frond in the specimen shown in Fig. 3 was 16 cm. long and $11\frac{1}{2}$ cm. broad near its upper end at its broadest.

The proliferations are generally elongated ellipsoidal and have often rather broad bases tapering from the middle towards the generally acute summit. Their size varies much from quite a small one up to about 2 cm. long and $\frac{1}{2}$ cm. broad or a little more.
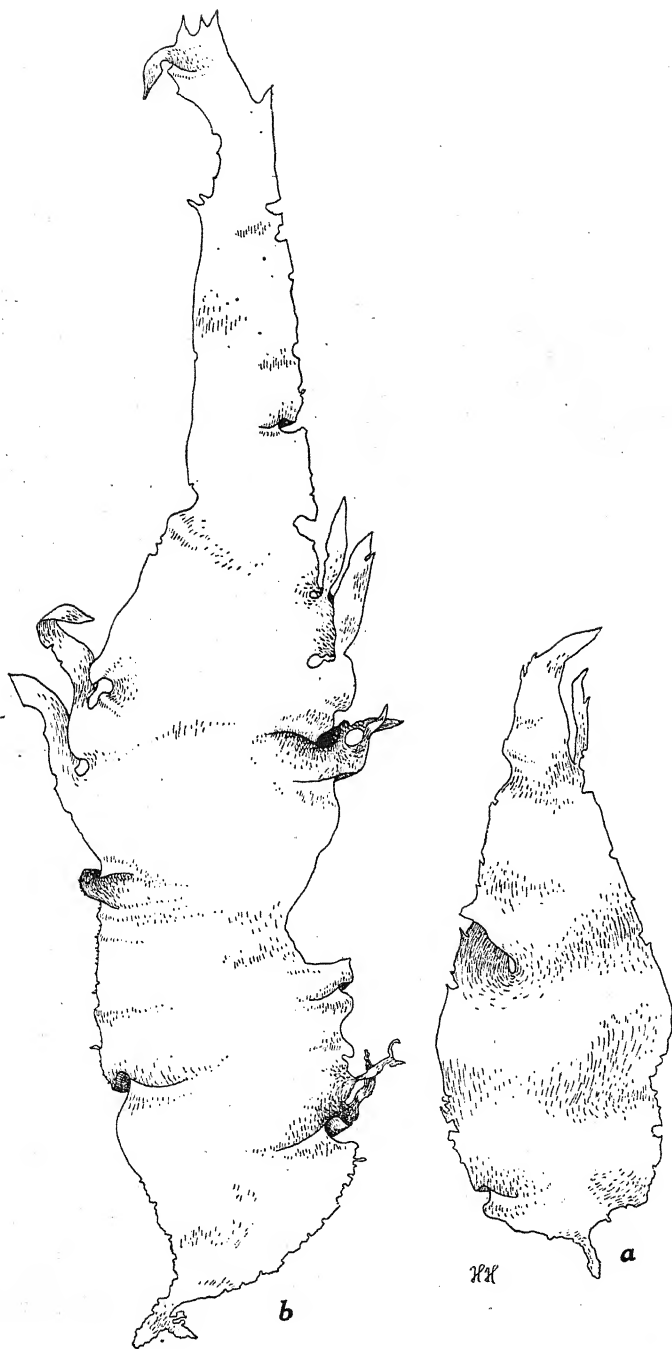
Fig. 4.—*Grateloupia* Comorinii Boergs.— about 3/5 natural size.

Judging from the fact that the plant adheres strongly to paper, the thallus must have been mucilaginous and slippery. The colour of the dried plant is brownish-purple with dirty yellow-greenish spots, especially in the basal parts. The sporangia are developed in the cortical layer and are scattered over the thallus; they are cruciately divided.

This species differs from *Grateloupia Cutleriæ* (Bind.) Kütz. (comp. De-Toni, Syll. IV, p. 1569) in having a different shape of the thallus and in the absence of proliferations on the surface of the thallus and also in the broadly cuneate base above the stipe. *Grateloupia Wattii* Holmes (in Journ. of Botany, vol. 38, 1896, p. 351) from Verawal, Kathiawar, India, differs according to the description in its palmately divided thallus having only a very few proliferations. The size of *Gr. Wattii* is not mentioned in the description. *Grateloupia indica* Boergs. (Kew Bulletin, 1932, 3, p. 119) differs in its much larger, oblong-linear thallus which often is much divided, in its often perforated thallus and also in its proliferations being formed only occasionally.

IV. Gigartinales

Fam. 1. **Solieriaceæ**

Sarconema Zan.

1. Sarconema furcellatum Zan., Pl. Mar. Rubr. Enum., p. 56, pl. 8.

A single rather robust specimen is perhaps referable to this species. Its thallus reaches a breadth of $1\frac{1}{2}$ mm. The filaments are only furcated a few times with long distances between the furcations. In the upper parts of some of the branches densely placed proliferations are present. The specimen is tetrasporic.

I n d i a : Cape Comorin, Sept. 1924, leg. M.O.P.I. (no. 296 A).

D i s t r.: Red Sea, Arabian Sea.

2. Sarconema filiforme (Sond.) Kylin, Die Florideenordnung Gigartinales (Lund's Univ. Årskr. N.F. Avd. 2. vol. 28, Nr. 8, p. 22, 1932).

A piece of a plant with repeatedly and regularly furcated thallus is most probably referable to this species; its thallus near the base is about 1 mm. thick tapering slowly upwards to about half that size. It resembles very much the specimens I have gathered at Bombay and referred to this species (Kew Bulletin 1934, p. 11).

I n d i a : Cape Comorin, Sept. 1924, leg. M.O.P.I. (no. 286 B).

D i s t r.: West Australia, Arabian Sea.

Fam 2. *Rhodophyllidaceæ*

Calliblepharis Kütz.

1. Calliblepharis Jubata (Good. et Woodw.) Kütz. Phycol. gen. p. 404; Tab. Phycol. vol. 18, pl. 13 a, b. J. AGARDH, Spec. Alg. II, p. 620; Epicr., p. 433—*Fucus jubatus* Good. et Woodw. in Linn. Transact. vol. III, p. 162, pl. 17, fig. 2, London 1797. For more literature compare DE-TONI, Syll. Alg., vol. 1V, p. 466.

In a collection of some Indian algae belonging to the University of Michigan and sent to me for determination by Professor W. RANDOLPH TAYLOR, Ann Arbor, a fine well prepared specimen is present showing a very great likeness to this plant.

In shape it agrees completely with specimens from the Atlantic shore of France collected for instance by THURET or LE JOLIS. The specimen is 24 cm. high and much divided with narrow, up to 7 mm. broad, segments up along the margins of which numerous proliferations are present; such proliferations are found on the flat sides of the thallus also. The specimen is tetrasporic. The zonately divided tetrasporangia are found both in the main lobes and in the proliferations, the plant in this respect seeming thus to have an intermediate position between *Calliblepharis ciliata* (Huds.) Kütz. in which species the tetrasporangia occur in the frond ("sphærosporis in fronde aggregatis," cfr. J. AGARDH, Epicrisis, p. 433) and *Calliblepharis jubata* where they occur in the proliferations (" sphærosporis in ciliis numerosis", cfr. J. AG., l c.). The specimen as mentioned above agrees in its shape so well with the last mentioned species that I have referred it to this species until more, also cystocarpic, specimens can be examined. But the occurrence of such a characteristic plant so far from its previously known area of distribution may of course arouse some doubt as to the correctness of its determination.

In the northern part of the Arabian Sea, *Calliblepharis fimbriata* seems to be common to judge from the rather numerous specimens I have seen in collections from that place, but I have not come across any specimens of *Calliblepharis jubata* there.

The plant was collected by E. W. ERLANSON at Trivandrum, Travancore. No dates are given, but ERLANSON together with E. K. JANAKI has gathered several other algae, *e.g., Chondria armata* in that locality in Jan. 1934 and the *Calliblepharis* was most probably collected in the same month.

D i s t r.: Atlantic shores of Europe from Scotland southwards to Morocco; Mediterranean Sea.

Fam. 3. **Hypneaceæ**

Hypnea Lamour.

1. Hypnea musciformis (Wulf.) Lamour. Cfr. Boergesen, Contributions, I, p. 47.

A few more specimens of this species most probably common along the Indian coasts are found in Iyengar's collection.

I n d i a : Cape Comorin, Pamban, leg. M.O.P.I.

Di s t r .: Most warm seas.

2. Hypnea valentiæ (Turn.) Mont. Cfr. Boergesen, l.c. p. 47.

Several other specimens of this highly variable plant are found in Iyengar's collection. Referring the reader to my remarks (l.c.), I wish to add that some of the specimens mentioned there, especially those referred with reservation to *H. spicifera*, might also be compared with *H. flagelliformis* Grev. collected by Wight at Madras. Of this plant I have seen specimens in J. Agardh's herbarium in Lund. Its numerous quite short branchlets which are protruding on all sides and covering the filaments quite densely are characteristic of this plant. But no specimens quite like those in J. Agardh's herbarium are found in Iyengar's collection.*

I n d i a : Tuticorin, Hare Island (!). Krusadi Island, Pamban, leg. M.O.P.I.

Di s t r .: Most warm seas.

Fam. 4. *Gracilariaceæ*

Gracilaria Grev.

1. Gracilaria confervoides (L.) Grev., Alg. Brit., p. 123. J. Ag., Spec. p. 587; Epicr., p. 413. Harvey, Phycol. Brit., t. 65.—*Fucus confervoides* L. Spec. plant., II, p. 1629. Turner, Fuci, tab. 84. For more literature comp. De-Toni, Syll. Alg., IV, p. 431.

Some specimens in Iyengar's as well as in my own collection are, I think, referable to this species. Specimens altogether typical I have not seen, and I think it is difficult to separate them from *Gracilaria lichenoides*.

I n d i a : Tuticorin, Hare Island, Febr. 29, female specimens, leg. M. O. P. I., (!); Krusadi Island, Apr. 1924, leg. M. O. P. I. ; Pamban, Oct. 1922, leg. M. O. P. I.

Di s t r . : Much distributed in warmer seas.

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*I wish to point out also that Wight at Madras has found another *Hypnea, H. nigrescens* (Grev.) J. Ag. (Alg. Liebmann, p. 14; Spec. Alg. vol. II, p. 443), No specimens of this species, which is represented in J. Agardh's herbarium, Lund, are found in Iyengar's collection.

2. Gracilaria disticha J. Ag., Spec. Alg., II, p. 594; Epicr. p. 416. *Sphærococcus distichus* J. Ag., Alg. Rueppel, p. 172.

A few specimens in IYENGAR's collection seem referable to this species. I have compared them with authentic specimens in J. AGARDH's Herbarium in Lund and found that they agree very well with them.

I n d i a : Krusadi Island, Pamban, May 1924, leg. M. O. P. I. and " Coll. by K. RANGACHARY " (without locality).

D i s t r .: Red Sea, India.

3. Gracilaria Fergusoni J. Ag., Spec. Alg., Vol. III, 4, p. 60, 1901.

A few specimens (one dried and some in formalin) seem to agree quite well with the description of this species based upon specimens gathered by FERGUSON on the shores of Ceylon. But I wish to point out that I have not been able to compare the Indian specimens with original ones.
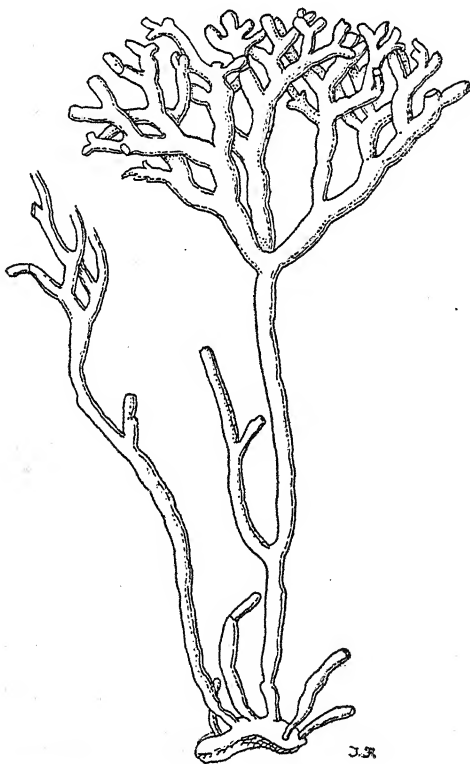
Fig. 5.—*Gracilaria Fergusoni* J. Ag. Habit of a plant. A little magnified.

The figure (Fig. 5) drawn from a specimen preserved in formalin shows a part of the plant. The plant grows most probably in exposed places in dense tufts about 6-7 cm. high. The base consists of a flat, more or less expanded disc. from which young as well as old erect shoots grow up intermingled. The thallus is terete, cylindrical, now and then somewhat narrowed, in the lower part about $2\frac{1}{4}$mm. thick tapering slowly a little upwards to about $1\frac{1}{2}$mm. at the upper ends; the apices of the branches are broadly rounded. As the ramification generally only begins at about half the height of the erect shoots the young ones up to this height are unramified. When the ramification begins the shoots are divided into two or three branches and these are once more pseudodichotomously divided several times, the branches growing in a fanlike manner in about the same plane and becoming shorter upwards.

A transverse section (Fig. 6) shows that the medullary layer is composed of rather small (about 80μ broad) cells with rather thick walls and of almost the same size until approaching the periphery where the cells become smaller and more oblong in transverse section. The cortical layer consists of quite small cells and is covered by a thick cuticle. The specimens were sterile.

When I tried to determine this plant, I began referring it to *Gracilaria obtusa* Grev. in Kützing, Tab. Phycol., vol. 19, p. 8, pl. 21; compare also Weber, Algues Siboga, p. 436. The fragments figured here by Kützing under the name of *Sphærococcus obtusus* might of course originate from a plant resembling the Indian plant, but this evidently cannot be the case, because one of the three specimens found under *Gracilaria obtusa* in Mme. Weber's Herbarium (in which Kützing's herbarium is incorporated) is a specimen of Harvey's Ceylon Algæ no. 30, and the two others were gathered at Ceylon by Schmarda. According to this statement these specimens must be presumed to be like *Corallopsis cacalia* Harvey = *Corallopsis opuntia* J. Ag., a plant which I am rather inclined to consider a form of *Gracilaria crassa* (compare my remarks in "Some Marine Algae from Ceylon," p. 86, fig. 8 and in "Contrib. to a South Indian mar. alg. Flora," II, p. 328-9). Kützing's figure quoted above agrees quite well with this species. To *Gracilaria obtusa* Mme. Weber refers also *Gracilaria canaliculata* (Kütz.) Sonder from Nova Caledonia and figured in Kützing's Tab. Phycol., vol. 18, pl. 82. This figure too, drawn from a rather fragmentary and badly preserved specimen might very well show a plant referable to *Gracilaria crassa*. Mme. Weber has also found that its thallus is really terete.

Owing to this I am very much inclined to believe that the rather dubious species *Gracilaria obtusa* Grev = *Sphærococcus*

obtusus (Grev.) Kütz.* from India and Ceylon is nothing but *Gracilaria crassa* (Harv.) J. Ag. From this plant *Gracilaria Fergusoni* is easily separated not only because of its quite different habit, but also because transverse sections of the two plants are very different, the medullary tissue of *Gr. crassa* consisting of cells more than twice as large as those of *Gr. Fergusoni* and having thin walls, making the thallus collapse very much, so as to give it a canaliculate appearance.
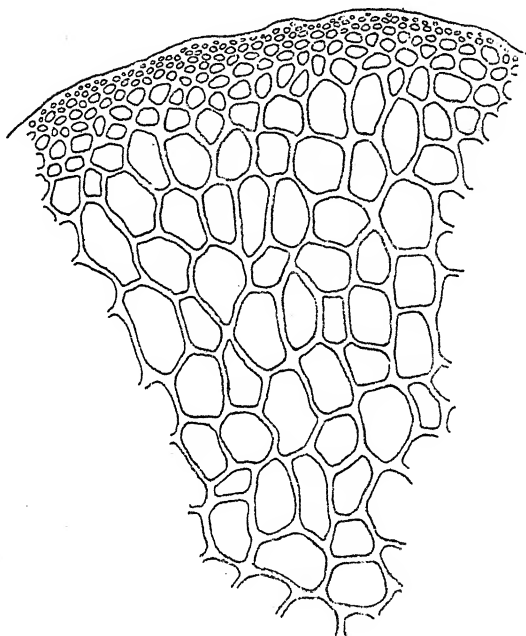

Fig. 6.—*Gracilaria Fergusoni* J. Ag. Transverse section of the thallus × 85.

Both IYENGAR and I gathered near Tuticorin a small form which, leaving its smaller size out of consideration, seems to agree very well in habit with *Gracilaria Fergusoni*. A transverse section shows that its cells are small, of about the same size as the big form.

I n d i a : Cape Comorin, Sept. 1924, M. O. P. I. Tuticorin, Oct. 1923, M. O. P. I. and March 1928 (!).

D i s t r.: Ceylon.

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* Compare also J. AGARDH, Spec. alg., vol. II, p. 590 Obs. and *Gigartina obtusa* (Grev.) J. Ag., Epicr. p. 426.

4. Gracilaria   debilis (Forssk.)   Boergs., Revision
Forsskåls Algæ, etc., p. 7.—*Gracilaria Wrightii* (Turner)
J. Ag., Spec. Alg., II, p. 599; Epicr. p. 422; III, 4, p. 78. *Fucus
Wrightii* Turner, Fuci, pl. 148.

It is not without doubt that I refer a female specimen in
Iyengar's collection to this species.   I have not been able to
compare it with specimens from the Indian Ocean, but, compared
to specimens from the West Indies, it has a somewhat thinner
and less fleshy thallus.   Otherwise the specimen seems to agree
quite well with the description of J. Agardh.

I n d i a : Krusadi Island.   Pamban, Oct. 1924, leg. M.O.P.I.

D i s t r .: Indian Ocean, Red Sea, West Indies.

5. Gracilaria corticata J. Ag., Spec. alg., II, p. 602;
Epicr., p. 423.—*Rhodomenia corticata* J. Ag., Symbolæ in
Linnæa, XV, p. 14, 1841.

It seems often rather difficult in the Indian material to
separate *Gracilaria corticata* from *Gracilaria foliifera*.   In Kew
Bulletin 1933, p. 124, I have tried to point out some of the
characters by means of which it might perhaps be possible to
separate these two species basing my point of view essentially
on J. Agardh's descriptions.   Later I have had the opportunity
to see the specimens of *Gr. corticata* in J. Agardh's Herbarium
in Lund.   Most of these have a narrow almost linear thallus ;
one of the plants with a somewhat broader thallus was a specimen
of Harvey's Ceylon Alg. no. 28.   This species was originally
described by J. Agardh from specimens from Ceylon, leg.
Reynaud (Herb. Paris).   These specimens I have not seen, but,
as they are from Ceylon and as J. Agardh refers Harvey's
Ceylon Alg. no. 28 to this species, it is to be presumed that this
plant comes near to the original specimens.   We have in the
Botanical Museum, Copenhagen, a specimen of Harvey's plant.
Its thallus is about 2 mm. broad tapering a little upwards, the
upper ends being obtuse.

In Iyengar's collection several specimens are, I think,
referable to this species.   Some of these are rather like Harvey's
above mentioned specimen, their thallus being rather regularly
subdichotomously divided with segments about 2 mm. broad and
with obtuse apices.   Other specimens are more irregularly
divided with more or less cuneate elongated segments tapering
gradually upwards and with acute apices resembling very much
Esper's figure, Tab. 50, in Abbildungen der Tange.   Finally
Iyengar's collection contains a few specimens with narrow
linear thalli like those found in Agardh's herbarium.

But, as I have already said, I find it difficult to separate this
species and especially forms with broad thalli from *Gracilaria
foliifera*.   I was therefore interested to find that Hauck in

Hedwigia, 1888, p. 89, has come to the same conclusion. He writes: "Sowohl in schmal–als auch in sehr breitlaubigen Formen vorkommend, welch letztere mit di-polychotom getheilten Thallus und bis 10-15 mm. breiten Segmenten sich gar nicht von der typischen *Gracilaria multipartita** unterscheiden lassen".

I n d i a: Karvar, Febr. 18th 1928, leg. M.O.P.I.; Tuticorin, Hare Island, March 2nd 1928, leg. M.O.P.I. (!); Cape Comorin, Sept. 1920, leg. M.O.P.I. In IYENGAR's collection presented to the British Museum some specimens from this locality are also found. Mahabalipuram, Jan. 1923, leg. M.O.P.I. Madras Harbour, Aug. 3rd 1924, leg. M.O.P.I.

D i s t r .: Indian Ocean, Red Sea.

6. Gracilaria foliifera (Forssk.) Boergs.

This species has already been mentioned in part I of these Contributions on page 48. A few more specimens in IYENGAR's collection are to be referred to it. Some of these resemble very much forma *æruginosa* Turner (*Fucus æruginosus* Turner, Hist. Fucorum, tab. 147). One of the plants (coll. by K. RANGACHARY, but without locality) is a rather slender form; in the British Museum (Nat. Hist.), London, some undetermined specimens resembling this form are found. Another specimen (cystocarpic) in shape and colour reminds one of forma *granatea* (*Fucus granateus* Turner, l. c. p. 215).

I n d i a: Cape Comorin, Sept. 1924, (f. *granatea*); Krusadi Island, Pamban, April 1924 (f. *æruginosa*), leg. M.O.P.I.

V. Rhodymeniales

Fam. 1. **Rhodymeniaceæ**

Rhodymenia Grev.

1. Rhodymenia dissecta nov. spec.

Frons plana, membranacea, ca. 20 cm. longa et ultra, basi cuneata, ad apicem versus sæpius dichotome-flabellatim divisa, ex segmentis sublinearibus ca. 3-8 mm. latis, axillis subacutis discretis, dichotomiis 2-6 cm. distantibus composita. Segmenta ad apicem versus plus minus angusta, apicibus obtusis vel subacutis. Segmenta basalia in marginibus prolications majores gerentia. In superiori parte thalli margines integerrimi sunt aut rarissime proliferi.

Tetrasporangia cruciatim divisa per totam superficiem thalli sparsa. Cystocarpia hemisphærica marginalia aut in disco thalli dispersa.

I n d i a: Tuticorin, Sept. 1900, leg. K. RANGACHARY. Specimina in Herb. IYENGAR and the British Museum (Nat. Hist.).

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* *Gracilaria multipartita* is a synonym of *Gracilaria foliifera*.

The thallus (Fig. 7) in the largest specimen is divided up to six times. The breadth of the segments varies as a rule from 3 mm. to ¾ cm. The angle between the segments is acute with a
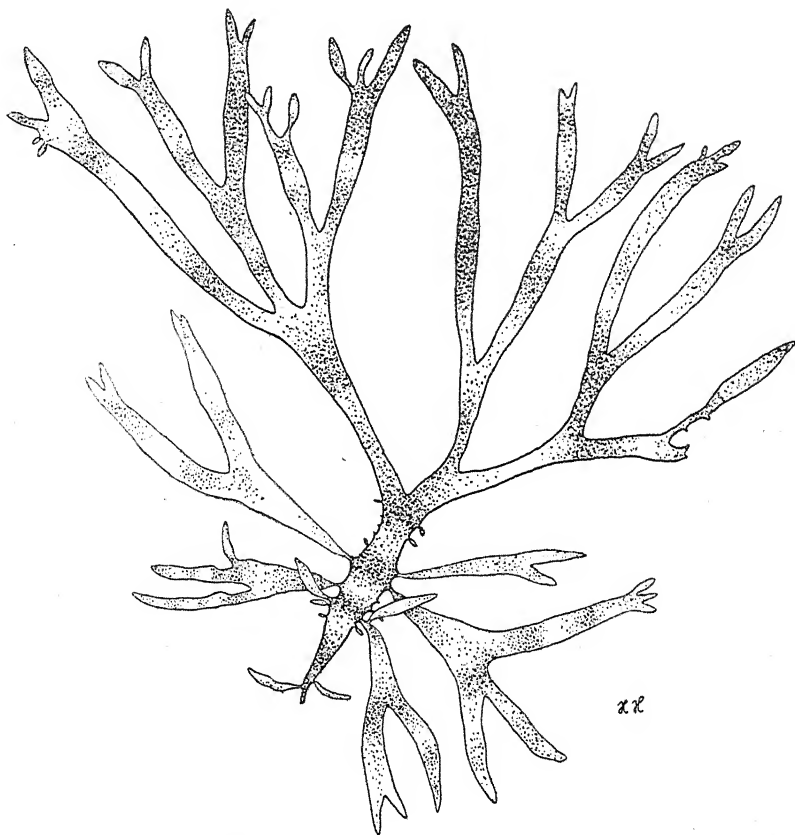
Fig. 7.—*Rhodymenia dissecta* Boergs.—about half the natural size.

more or less roundish base, and the distance between the dichotomies is 2-6 cm. The plant is fixed to the substratum by means of a small disc from which the elongated cuneate basal segments arise along the margins of which on both sides a row of proliferations (3-5 in each row) are developed. These proliferations are divided as the main thallus but only about twice, and their segments reach about the same breadth. Altogether I have seen two large tetrasporic specimens and a small female one of this plant and also a small specimen which I am not sure belongs to this species. In the large specimens the margins above the basal segment are smooth and without proliferations except for a

few small ones. The small specimen, on the other hand, not only had very many small proliferations along the basal segment, but such ones also occurred in a scattered manner, higher up in the plant but in decreasing numbers. The thallus is thin, papery and brittle when dried. I have not succeeded in making it assume its natural shape by soaking it in water. Except in the case of the small specimens, it does not adhere very well to paper. The colour of the dried specimens is dirty red-violet to brownish. Three of the specimens are tetrasporic; the tetrasporangia occur scattered over the whole thallus. The fourth specimen is a female one and the cystocarps are likewise developed scattered over the whole thallus. The two bigger specimens of which one (Fig. 7) belongs to the herbarium of the British Museum (Nat. Hist.), Coll. EDGAR THURSTON, and the other one to Prof. IYENGAR, have both no. 24 and are tetrasporic and have been collected Sept. 15th 1900. The small female specimen belongs to IYENGAR's herbarium and has no. 37 and is dated Sept. 16th 1900, and from the same date is the small somewhat different specimen which has no. 36 and belongs to the British Museum.

Since the tetrasporangia are scattered over the whole thallus, this species belongs to the group *Palmatæ* J. Ag., Spec. Alg. p. 376, but judging from the descriptions of the species referred to this group, it seems to be related to none of them.

VI. Ceramiales

Fam. 1. *Ceramiaceæ*

Sub-fam. 1. *Griffithsieæ*

Griffithsia C. Ag.

1. Griffithsia spec.

Several sterile specimens are found in IYENGAR's collection. They remind one very much of KÜTZING's figure of *Griffithsia opuntioides* in Tab. Phycol. Vol. XI, pl. 27, but as they are sterile they are undeterminable.

India: Cape Comorin, Sept. 1924, leg. M.O.P.I.

Sub-fam. 2. *Ceramieæ*

Ceramium (Roth) Lyngbye

In IYENGAR's collection I have occasionally come across in the bottles some pieces of *Ceramium* as epiphytes on larger algae or mingled with these. Dr. H. E. PETERSEN has been so kind as to identify them and the result is the following list.

1. Ceramium strictum Grev. et Harv. in HARVEY, Phycologia Brit., pl. 334. J. Agardh, Spec. alg., vol. II, p. 123; Epicr., p. 97.

Some small sterile fragments only are found.

I n d i a : Cape Comorin, Oct. 1924, leg. M.O.P.I.

D i s t r.: Warm parts of the Atlantic Ocean, Mediterranean Sea.

2. Ceramium cruciatum Collins and Herv., Alg. Bermuda, p. 144, pl. IV, figs. 27—28. WEBER VAN BOSSE, Algues Siboga, p. 331, fig. 122.

Tetrasporic specimens are found in one of the collections.

I n d i a: Krusadi Island, Pamban, Oct. 1924, leg. M.O.P.I.
D i s t r. West Indies, Malayan Archipelago.

3. Ceramium transversale Collins & Herv., Alg. Bermuda, p. 145, pl. V. figs. 29—31. BOERGESEN, Mar. Alg. D.W.I., vol. II, p. 243, fig. 233; Marine Algae Beata Island, p. 27, fig. 9. —*Ceramium byssoideum* Harv., HOWE in BRITTON, Flora of Bermuda, Algae, p. 531. *Ceramium gracillimum* Griff. et Harv., Kew Bulletin, 1934, p. 19.

When Dr. H. E. PETERSEN in 1934 was kind enough to determine the species of *Ceramium* in my Indian collection, he was at that time inclined to consider *Ceramium transversale* to be like *Ceramium gracillimum* Griff. et Harv., but added that authentic material was necessary in order to be quite certain regarding this. Recently Dr. PETERSEN has been able to say with certainty that *Ceramium gracillimum* is quite a different plant, having for instance gland-cells which are not found in *Ceramium transversale*.

A few small specimens are found in IYENGAR's collection.

I n d i a: Royapuram, Madras, Aug. 1924, leg. M.O.P.I. As mentioned in Kew Bull. 1934, I have found this species near Tuticorin.

D i s t r.: Seems to occur in most warm seas.

4. Ceramium truncatum H. E. P. in BOERGESEN, Mar. Algae from Ceylon (Ceylon Journ. of Sc., Botany, vol. XII, 1936, p. 91, fig. 11, 12).

Tetrasporic specimens were found as epiphytes on leaves of *Sargassum*.

I n d i a: Cape Comorin, Oct. 1924, leg. M.O.P.I.
D i s t r. Ceylon.

5. Ceramium subdichotomum Web. v. B., Algues Siboga, p. 333, fig. 125.

Tetrasporic specimens are met with in some of the collections.

I n d i a : Tuticorin, Hare Island, Febr. 1928, leg. M.O.P.I. Krusadi Island, Pamban, April 1924, leg. M.O.P.I.

D i s t r.: Malayan Archipelago.

6. Ceramium Maryae Web. v. B., Algues Siboga, p. 324. figs. 117 and 118.

A single sterile specimen was found creeping on *Enantiocladia prolifera.*

I n d i a : Cape Comorin, Oct. 1924, leg. M.O.P.I.

D i s t r.: Malayan Archipelago.

Fam. 2. Rhodomelaceæ

Sub-fam. 1. Laurencieæ

Laurencia Lamour.

1. Laurencia paniculata J. Ag., Spec. Alg., p. 755 ; Epicr. p. 651. YAMADA, Notes on Laurencia, p. 192, pl. 3, fig. a, 1931.

In IYENGAR's collection two small specimens are found which resemble very much the small specimens from Ceylon referred by me to this species in " Some marine Algae from Ceylon " 1936, p. 93. The peripheral cells have the shape of palisades. These specimens came from pearl-beds near Tuticorin.

Furthermore, a few somewhat larger specimens from Cape Comorin are perhaps referable to this species. The peripheral cells of these specimens also are palisade-like with thick walls. IYENGAR has presented some specimens collected from this locality to the British Museum.

I n d i a : Pearl-bed near Tuticorin, March 3rd, 1928 and Cape Comorin, Sept. 1920, leg. M.O.P.I.

D i s t r.: Mediterranean sea, Ceylon.

2. Laurencia obtusa (Huds.) Lamx.

Several specimens varying in form, size and development are found in IYENGAR's collection, but as a rule only a single specimen of each form is present. One of the specimens seems to belong to the var. *divaricata* J. Ag. resembling very much the figure of the type-specimen in J. Agardh's herbarium figured in YAMADA's paper: Notes on Laurencia, pl. 16 a. Another one, I think, is referable to the var. *majuscula* Harv., resembling the plant mentioned in Kew Bulletin 1933, p. 135, and having the same fine red colour. Compare also YAMADA's figure l. c. pl. 16 c,

A small slender plant is very much like var. *gracilis* Kütz., Tab. Phycol., vol. 15, p. 20, tab. 54. figs. c, d. Fig. 8 shows a piece of this plant. Thickened walls are not present in the medullary layer. I dredged this plant at a depth of about 20 meters. It had tetrasporangia in March.
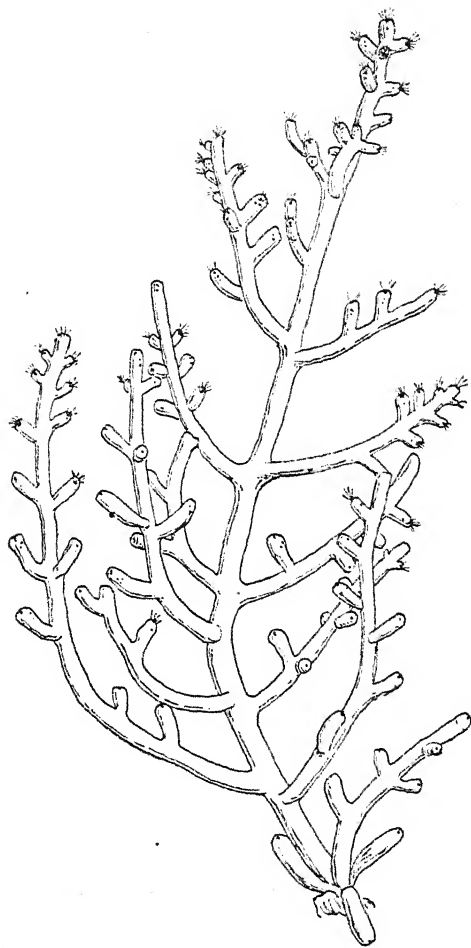
Fig. 8.—*Laurencia obtusa* (Huds.) Lamx. var. *gracilis* Kütz. Part of a plant × 5.

Furthermore, a small incomplete specimen shows much likeness to the var. *rigidula* Grunow, Alg. Fidschi, Tonga und Samoa-Inseln, p. 45. The divaricate, as a rule oppositely or verticillately placed branchlets are characteristic of this variety. It shows, as mentioned by Grunow, very great likeness to

Kützing's figure of *Laurencia corymbifera* from the West Indies in Tabulæ Phycol., vol. XV, tab. 56. The specimen is collected by K. Rangachary, but the locality is not mentioned.

India: Off Tuticorin (!), Cape Comorin and Pamban Bridge, leg. M.O P.I.

Distr.: Warmer Seas.

Sub-fam. 2. *Chondrieæ*

Chondria Ag.

1. Chondria transversalis nov. spec.

Thallus ca. 1-2 cm. altus, filiformis, teretiusculus, ad 450μ latus, ramosus ex ramis decumbentibus, rhizoideis adfixis et ramis erectis compositus. Apex ramorum truncatus, punctum vegetativum in foveam crateriformem immersum. Ramificatio subpyramidalis, ramis sparsis, clavatis, ca. 250μ latis inferne tenuioribus, superne subcylindricis trichoblastos numerosos
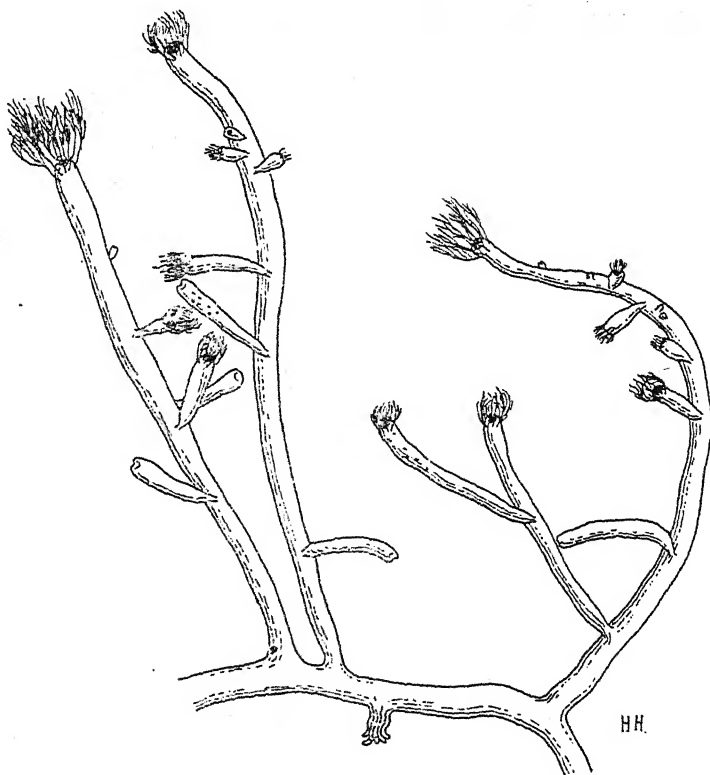
Fig. 9.—*Chondria transversalis* Boergs. Habit of plant. $\times$ 9.

gerentibus. Cellulæ epidermales elongatæ-subquadranguleres ad 140µ longæ et ca. 30µ latæ; cellulæ subepidermales oblonge-hexagonæ, ca. 140µ longæ et ca. 15µ latæ in series transversales regulares ordinatæ.

Tetrasporangia in superiore parte ramorum præsentia.

I n d i a : Off Tuticorin near Hare Island dredged at about 20 meters on 1st March 1928, Boergesen 5737 (type).

This little *Chondria* (Fig. 9), creeps on stones and shells in rather deep water. The thallus is terete and composed of decumbent creeping filaments from which the erect ones arise. The creeping filaments are fixed to the substratum by means of short, thick rhizoids. The thickest thallus found was about 450µ broad, but the most common breadth is 350-400µ, the branches reach a breadth of about 250µ. The erect filaments are up to 1½ cm. high. The ramification is pyramidal, branches being given out on all sides from the main branches. The branches at their base are narrowed often to almost half their size, upwards they are cylindrical keeping nearly the same size until the truncate or broadly rounded apex with the sunken growing point in the middle from which the often numerous trichoblasts protrude. The surface cells (Fig. 10a.) when seen from above are elongated
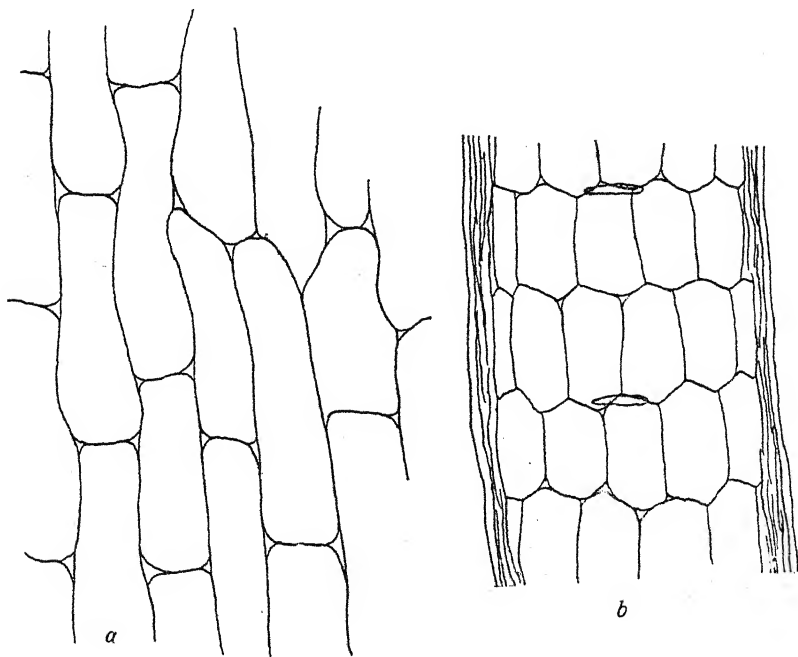
Fig. 10.—*Chondria transversalis* Boergs. *a*, cortical cells seen from above. *b*, subcortical. cells *a*, × 265. *b*, × 90.

quadrangular, up to about 140μ long and 30μ broad. The cells
under the surface cells are clearly observable. They are elongated
hexagonal (Fig. 10b) about 140μ long and 75μ broad and arranged
in regular transverse rows. Also the central cells and especially
the thick transverse walls of these are visible. The central cell
and the pericentral ones are twice as long as the subcortical cells
forming the transverse rows. A transverse section shows
(Fig. 11) in the middle the central cell and 5 pericentral cells;
these are surrounded by the large subcortical cells which are in
their turn surrounded by the peripheral cells which are
roundish-quadrangular in transverse section and are firmly
connected with one another in contrast to the cells of the interior
which are more loosely connected with one another. The tetras-
porangia are formed in the upper ends of the branches.
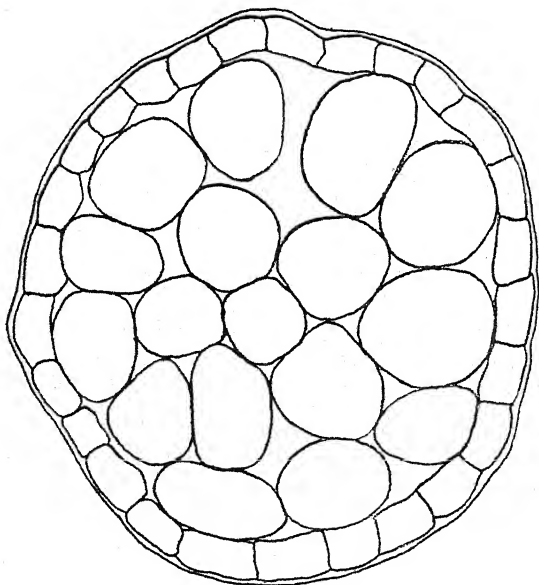
Fig. 11.—*Chondria transversalis* Boergs. Transverse section of the
thallus $\times 265$.

This species seems to resemble very much *Chondria sim-
pliciuscula* Weber in Rhodophyceae of the Percy Sladen Trust
Expedition (Transactions of the Linnean Soc. of London, Zoology,
vol. 16, 1914, p. 289, pl. 16, figs. 9-10), although, judging from the
description and rather schematic figures, it differs in several
respects. The Indian plant agrees with *Ch. simpliciuscula* in
being small (exact measurements of the latter are not given)
and in having a decumbent creeping thallus. Furthermore, the
hexagonal shape of the cortical cells near the apices of the

filaments is about the same in both species and the tetrasporangia are formed in the upper ends of the ordinary branches. As regards the differences between the two plants, it must be mentioned that in the old parts of the thallus in Mme. WEBER's plant the superficial cells have undulated walls and become thus moniliform, whereas, in the Indian plant, these cells are long and subrectangular with straight walls. Further, Mme. WEBER's plant is an epiphyte creeping on *Laurencia papillosa* and thus must be presumed to live in shallow water while the Indian plant was found in deep water fixed by means of rhizoids to small pieces of stones and shells. Besides, as the description given above clearly shows, the Indian plant differs essentially from *Ch. simpliciuscula* in several other respects also.

Sub-fam. 3. **Polysiphonieæ**

Polysiphonia Grev.

Polysiphonia Tuticorinensis nov. spec.

Cæspes erectiusculus ca. 12 cm. altus, fastigiate-virgatuss fuscopurpureus. Fila ecorticata ex articulis 4-siphoneis composita, a basi ramosa ramos sparsos quoqueversum egredientes, erectos, axillis acutis gerentia, inferne ca. 400μ lata, moniliformia, superne gradatim tenuiora et cylindrica. Articuli in tota planta conspicui sæpe paulo atiores quam longiores. Rami in loco pilorum oriuntur.

Tetrasporangia pauca (1-3) in ramulis torulosis spiraliter seriata in superiore parte filorum præsentia; segmenta fertilia, ca. 70μ lata, tetrasporangia 50μ lata.

Cystocarpia oblonge-urceolata, ca. $250-280\mu$ longa et $180-250\mu$ lata, in superiore parte cellulis permagnis ostiolum apicalem cingentibus ornata.

Antheridia elongate-conica, ca. 160μ longa et 40μ lata. superne cellulas steriles 1-3 gerentia.

I n d i a: Hare Island, 2nd March 1928, BOERGESEN 5764 (type).

The plant (Plate VIII) forms a dense very much ramified tuft about 12 cm. high. Its method of growth is pyramidal with scattered upwardly erect branches all along the main filaments. The plant has four pericentral cells and no cortical layer. In the lower part the main filaments are about 600μ broad or more (?), tapering slowly upwards. In the older lower parts of the thallus the filaments are moniliform, but higher up they are cylindrical; the segments are about as long as broad or a little shorter, for instance, in one filament with a breadth of 352μ the segments were 242μ long, and another filament which was 275μ broad had segments about 220μ long. The trichoblasts are well developed

in the young upper parts of the filaments (Fig. 12a); they are placed spirally to the left with $\frac{1}{4}$ circumference between them in such a way that the 5th trichoblast is situated over the first one. Now and then a branch is given off instead of a trichoblast.
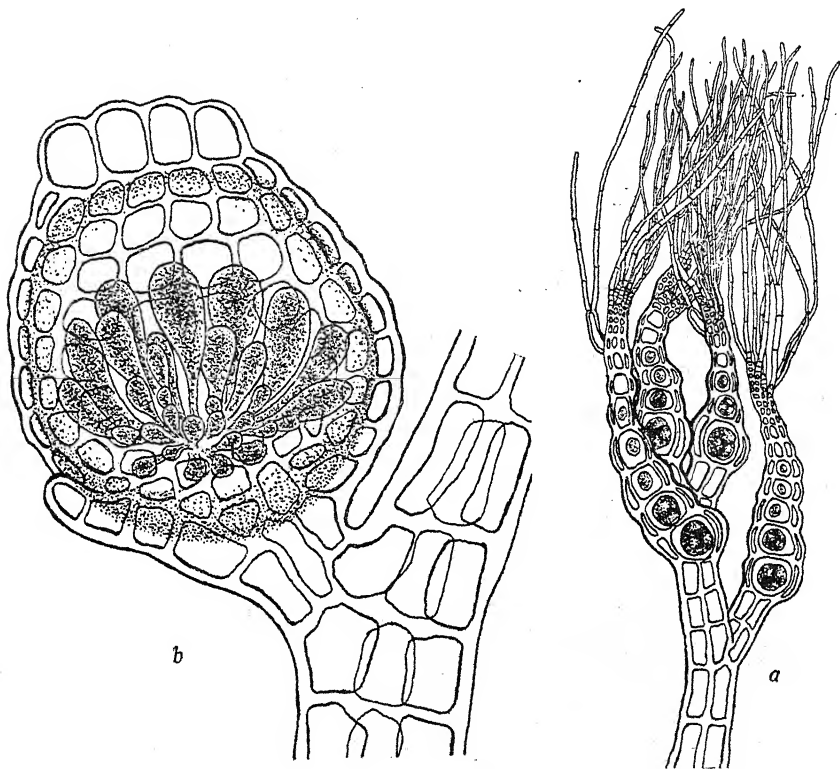

Fig. 12.—*Polysiphonia Tuticorinensis* nov. spec. *a*, filaments with tetrasporangia *b*, a cystocarp. *a* × 70; *b*. × 135.

The tetrasporangia (Fig. 12a) are developed in short rows. As a rule 2 (1-3) well developed tetrasporangia are found and above these a few badly developed ones. One sporangium is developed in each segment. These become swollen very much making the branch more or less screw-shaped. The fertile segments reach a breadth of 70µ and the sporangia a breadth of about 50µ.

The cystocarps (Fig. 12b) are elongated-urceolate about 250-280µ long and 180-240µ broad, the neck is rather long and about 115-130µ broad, and the large cells forming uppermost a ring round the ostiole are about 20µ high.

The androphores (Fig. 13) are formed by the first side-branch of the trichoblasts. They are elongated conical about 160μ long and 40μ broad and have at their upper ends 1-3 short sterile cells. The only male branch found by me was a side branch on a tetrasporic plant. Kniep in "Die Sexualität der niederen Pflanzen" mentions p. 226 several similar cases found in *Polysiphonia*.
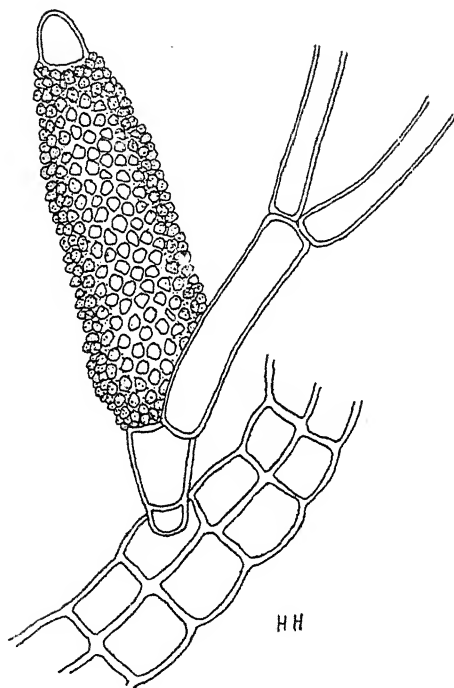
Fig. 13.—*Polysiphonia Tuticorinensis* nov. spec. An androphore × 400.

Polysiphonia Tuticorinensis compared with the species of *Polysiphonia* from India previously described by me is easy to recognize. Thus *Polysiphonia platycarpa* (in Kew Bull., 1934, p. 23, figs. 15-17 and in List mar. Alg. Bombay, p. 60) differs owing to the facts that the cystocarps are more spherical in shape and have no large cells round the ostiole, the androphores have no sterile cells at their summits, and the tetrasporangia are found in long rows. To this must be added the altogether different habit of the plant. *Polysiphonia unguiformis* (Contributions, I, p. 53) differs in the absence of large cells round the ostiole of the cystocarps and in the androphores not having large sterile cells at

3

the upper ends.   The ramification and habit of this plant is also quite different.

Owing to the often very incomplete descriptions without figures of many of the almost 50 species with four pericentral cells mentioned in DE-TONI's Sylloge, I dare not deny that some of these might possibly come near to or perhaps be like the present species, but I wish to point out that I have gone through the collections of *Polysiphonia* found in the Kew Herbarium and in British Museum without being able to find any specimens resembling this species.

F. BOERGESEN—*GRATELOUPIA LITHOPHILA* BOERGS.

F. BOERGESEN—*POLYSIPHONIA TUTICORINENSIS* BOERGS.
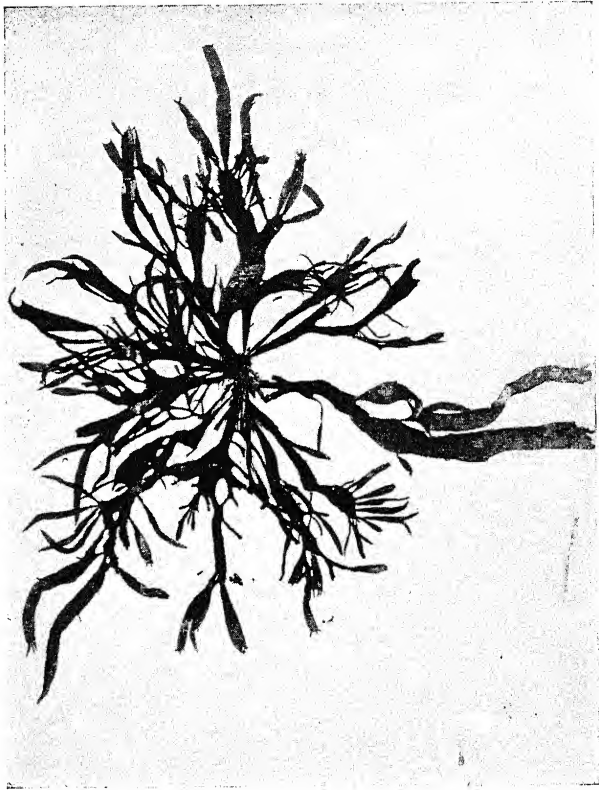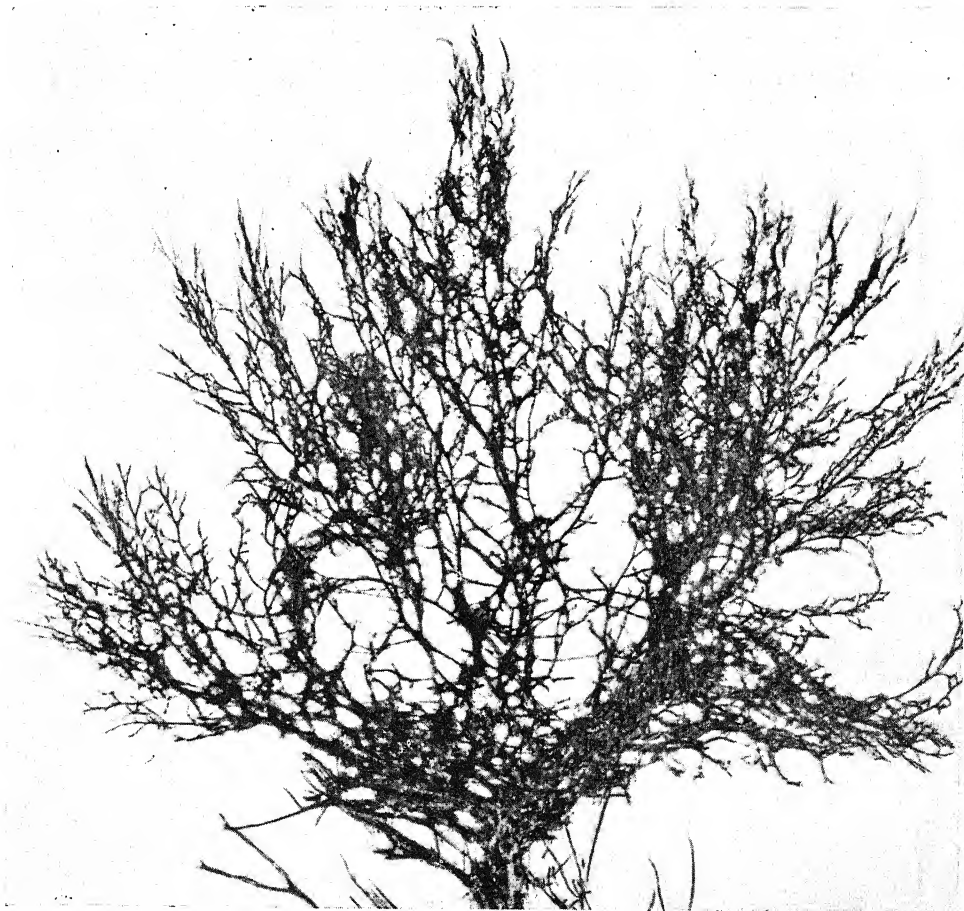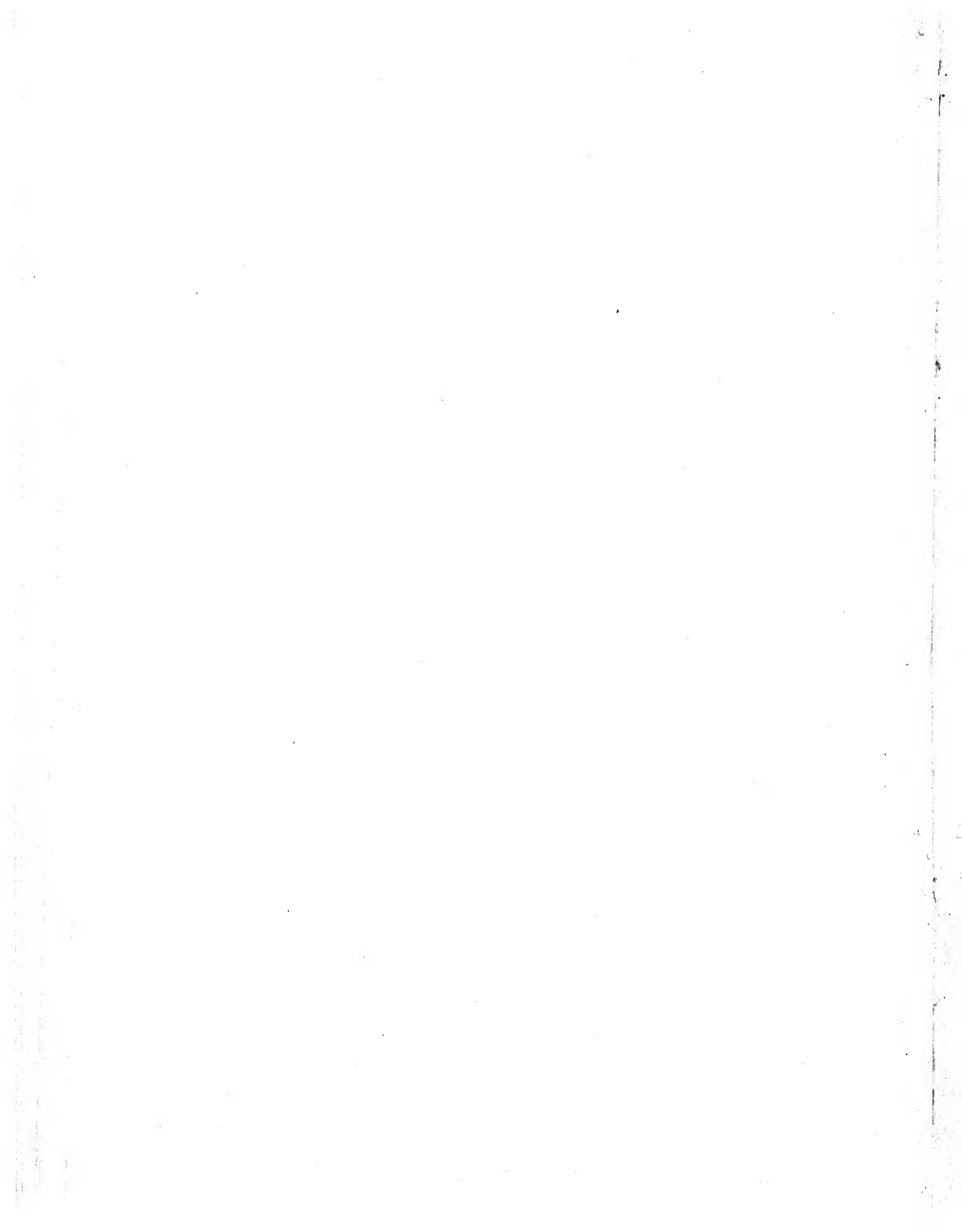
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EMBRYO AND SEED DEVELOPMENT IN THE NYCTAGINACEAE
I. STUDIES IN THE GENUS *BOERHAAVIA*

BY

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Communicated by A. C. Joshi

Introduction

The present paper deals with the various stages in the development of the embryo and seed of *Boerhaavia diffusa* Linn. and *Boerhaavia repanda* Willd., two members of the family Nyctaginaceae found in the Gangetic plain and other parts of India. The development of the embryo-sac and the pollen in these plants has already been described by Maheshwari (1929) and Bhargava (1932). They have also made a few observations on the embryo development, but these are of a fragmentary nature, and do not provide a correct picture of the same as pointed out by me in a preliminary note (Kajale, 1936).

Out of the two plants described here, *Boerhaavia diffusa* has been studied in greater detail than the other. Both the plants, however, resemble each other very closely. A common account, therefore, has been written, the few differences in details being pointed out at the proper places.

The Ovule

The carpel in these two species of *Boerhaavia,* as in the Nyctaginaceae in general, possesses a single basal ovule. This is provided with a short funicle. The form of the ovule has been described by Maheshwari (1929) as anatropous. The material examined by me, however, shows that there is a distinct bend in the ovule on the side of raphe, pointing towards campylotropy. The figures of Maheshwari and Bhargava also show this clearly. The ovule thus has the same form as in *Gisekia pharnaceoides* studied by Joshi and Rao (1936), and should be described in the same way as ana-campylotropous.

Another point missed by Maheshwari (1929) and Bhargava (1932) is about the development of the epidermal cap at the micropylar end of the nucellus. Figures 29 and 32 of Maheshwari and 39 and 41 of Bhargava show clearly the development of such cap,

but the authors have not referred to this point in the text. The presence of the nucellar epidermal cap in these forms tends to bring the Nyctaginaceae into line with the other Centrospermales, for the development of such a cap seems to be a characteristic feature of this order.

In *Boerhaavia repanda,* Bhargava (1932) has described the formation of a mucilaginous mass near the micropyle of the ovule. Such a structure is seen in *Boerhaavia diffusa* also. It is, however, nothing more than the continuation of the transmitting tissue of the carpel. Similar tissue is also seen in *Mirabilis Jalapa* and Guéguen (1901) had long ago worked out its true nature.

The Development of the Embryo

The first division in the fertilised egg is by a transverse wall and leads to the differentiation of an apical and a basal cell (Fig. 3). The next division takes place in the basal cell (Fig. 4), and as again transverse. In this manner a filamentous proembryo of three cells is formed (Figs. 5, 9 & 20). The proembryo may remain in this condition till the appearance of the first longitudinal wall or another transverse division may take place in the cell at the micropylar end, and the proembryo may become four cells long before the apical cell divides longitudinally (Fig. 6). Further increase in the length of the embryo takes place always after the appearance of a longitudinal wall in the apical cell, and again is the result of a transverse divison in the micropylar cell. Thus every increase in the length of the embryo up to this stage is due to repeated divisions in the micropylar cell. This is clearly brought out by figure 1.
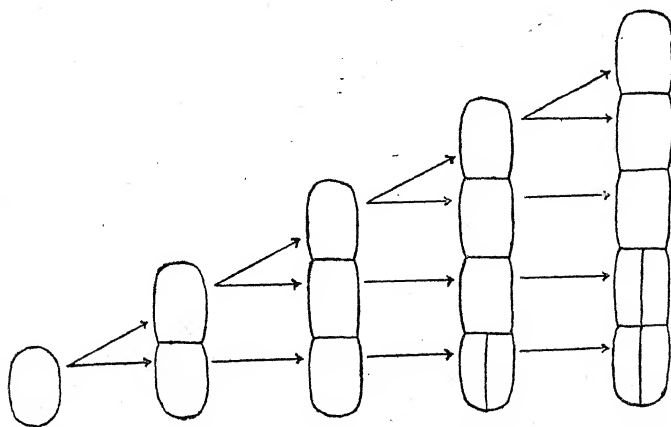

Fig. 1. A diagramatic sketch of the development of embryo till it has become 5 cells long, showing the derivation of various cells by repeated divisions in the basal cell.

The embryo proper develops from four apical cells of the proembryo, but the time of differentiation of these four cells is peculiar as compared with the members of the Amarantaceae (Joshi and Kajale, 1937), Chenopodiaceae (Souèges, 1920), Caryophyllaceae (Souèges, 1924), etc. In the latter families these cells are all formed and can be counted before any longitudinal division begins in the cells of the proembryo. In the plants under investigation one or two of the lower cells, depending upon the total number of cells of the proembryo, are not even formed when the first longitudinal division takes place in the apical cell. These cells are cut off only afterwards from the micropylar cell. Figures 7, 8 and 9 from *Boerhaavia diffusa* and figure 21 from *Boerhaavia repanda* show this point clearly. In figure 9 for instance the apical cell has divided longitudinally into two. The penultimate one is dividing in the same manner. The third cell which has to take part in the development of the embryo proper is just differentiating, while the fourth one is still to be cut off.

The differentiation of the embryo from these four cells is as follows.: the apical cell forms the cotyledons and the plumule. The second and third cell from the apex give rise to the hypocotyl and the greater part of the radicle. The fourth cell forms the hypophysis, from which the apex of the radicle is completed.

Generally four cells only are concerned in the development of the embryo. A few cases in *Boerhaavia repanda,* however, have shown that some times five cells may take part in its development. Figures 27 and 28 provide two such illustrations. The accessory cell takes part in the development of the hypocotyl and radicle.

As the embryo reaches a length of three or four cells, longitudinal walls begin to appear. The first longitudinal division takes place in the apical cell (Figs. 7, 8 & 21). It is this feature which has probably led Maheshwari (1929) to conclude that the embryo development in *Boerhaavia diffusa* corresponds to the *Capsella*-type. Next such a wall appears in the penultimate cell (Figs. 9, 21 & 22), and this is followed by a longitudinal division in the third cell from the apex (Figs. 10 & 23). This sequence of the appearance of longitudinal walls is in direct contrast with what is seen in other investigated members of the Centrospermales, e.g., *Chenopodium Bonus-Henricus* (Souèges, 1920), *Alternanthera sessilis* and *Digera arvensis* (Joshi and Kajale, 1937), *Achyranthes aspera* (Kajale, 1937), *Gisekia pharnaceoides* (Joshi and Rao, 1936), and *Sagina procumbens* (Souèges, 1924). In all these examples the first longitudinal division takes place either in the second or third cell from the apex, and then only it proceeds towards the apical cell.

As the longitudinal division takes place in the third cell or soon after its completion, the apical cell undergoes one more longitudinal division in a plane at right angles to the first, and thus gives rise to
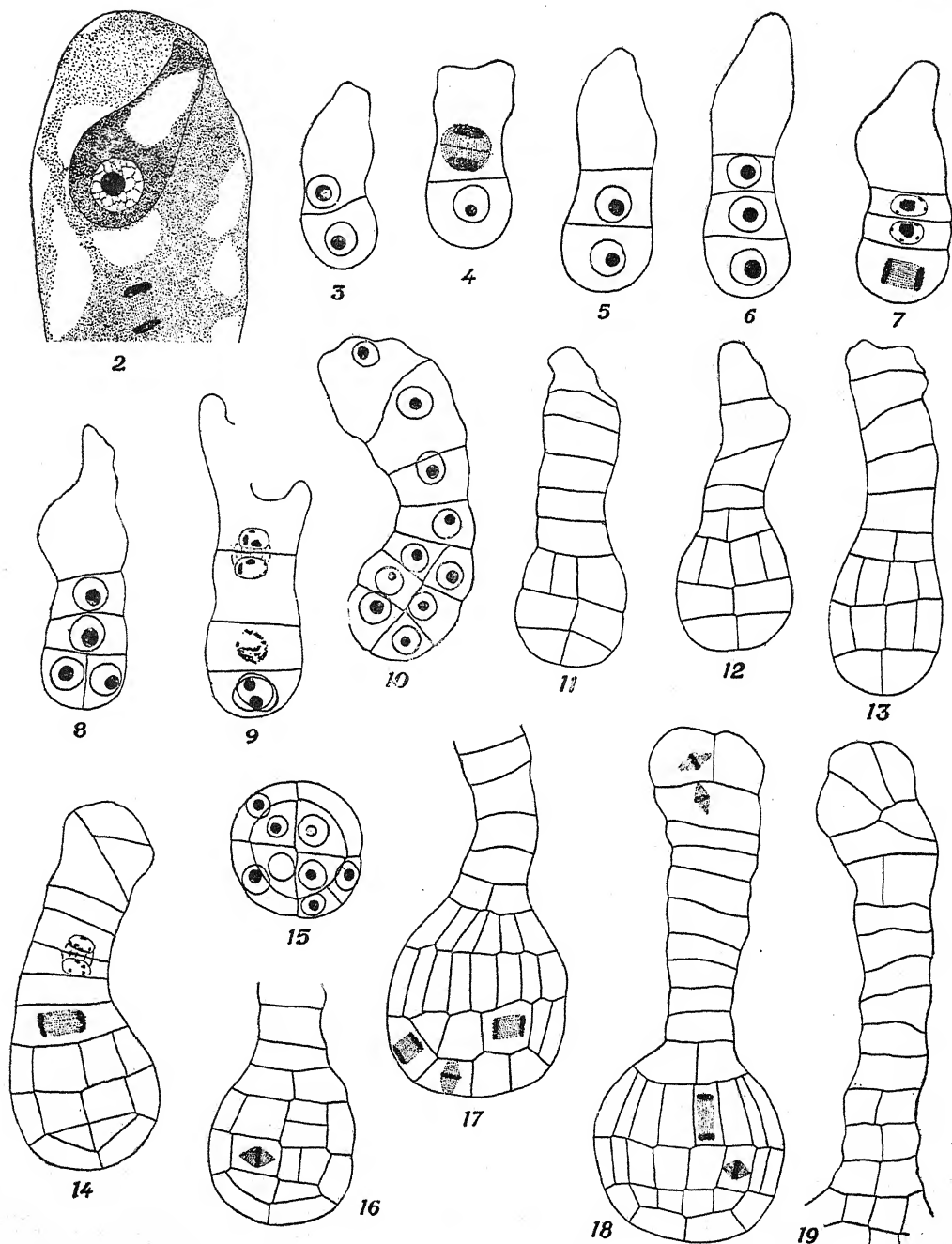
Figs. 2-19. *Boerhaavia diffusa*. Fig. 2, micropylar part of an embryo-sac showing the fertilised egg and the dividing endosperm nucleus. Figs. 3-18, various stages in the development of the embryo. Fig. 15 represents a transverse section of one of the three apical cells and shows the formation of quadrants. In Figs. 16 & 17 micropylar part of the suspensor is not shown. Fig. 19 shows the structure of the suspensor. For further explanation see text. × 450.

the quadrants (Figs. 15 & 23). This is followed by the organisa-
tion of quadrants in the second and third cells.

After the quadrants have been organised in the three tiers each
cell divides by a periclinal wall. Thus the dermatogen differentiates.
It first appears in the third tier from the apex, next in the second,
and finally in the apical tier (Figs. 11 to 14 & 24 to 26).

After the dermatogen has been completed, the differentiation
of the two other histogenic layers begins. This proceeds in a very
interesting way and differs markedly from what is seen in other
angiosperms. Generally in the flowering plants the dermatogen cells
after their differentiation divide only by anticlinal walls, so that
this layer may keep pace with the growing embryonal mass. In
species of *Boerhaavia,* however, these cells also divide once peri-
clinally (Figs. 17, 18, 27 & 28). The resultant inner cells give rise
by further periclinal divisions to the periblem of the embryo. The
cells cut off towards the outside do not divide any further pericli-
nally, and appear to behave from now onwards like the dermatogen
of other angiosperms. The inner cells cut off by the first periclinal
divisions in the quadrants differentiate into the plerome of the
embryo (Fig. 18). The rest of the development of the embryo,
including the development of the hypophysis, is quite similar to that
of *Chenopodium Bonus-Henricus* as described by Souèges (1920).

The suspensor develops from the micropylar cell formed after
the hypophysis cell has been cut off, i.e., from the sister cell of the
hypophysis. Its structure differs in the two species of *Boerhaavia.*
In *Boerhaavia diffusa,* it is a long and slender structure. Ten to
thirteen cells can be counted in its length, but through its greater
part it is only one or two cells broad (Figs. 18 & 19). The suspensor
of *Boerhaavia repanda* differs from that of *Boerhaavia diffusa* in
being shorter in length but more massive (Figs. 29 & 30). It thus
seems to agree with that of *Mirabilis Jalapa* described by Woodcock
(1929). I can not bear out the statement of Bhargava (1932) that
in *Boerhaavia repanda* "sometimes the suspensor has two rows of
cells at places and occasionally even in older stages remains
uniseriate."

Mature Embryo

The mature embryo is not exactly annular as in many other
Centrospermales but is bent over the columnar perisperm (Fig. 32).
The bend exists in the region of the hypocotyl. The radicle and the
cotyledons consequently are straight and nearly parallel in longitudinal
section. The cotyledons in transverse section appear more or less
concave (Fig. 33), and the inner one is seen to be smaller than
the outer. They are further characterised in *Boerhaavia repanda*
by the presence of certain cells, which are bigger than the rest and
contain raphides of calcium oxalate. These raphides are absent
from the hypocotyl and the radicle, and are not found at all in any
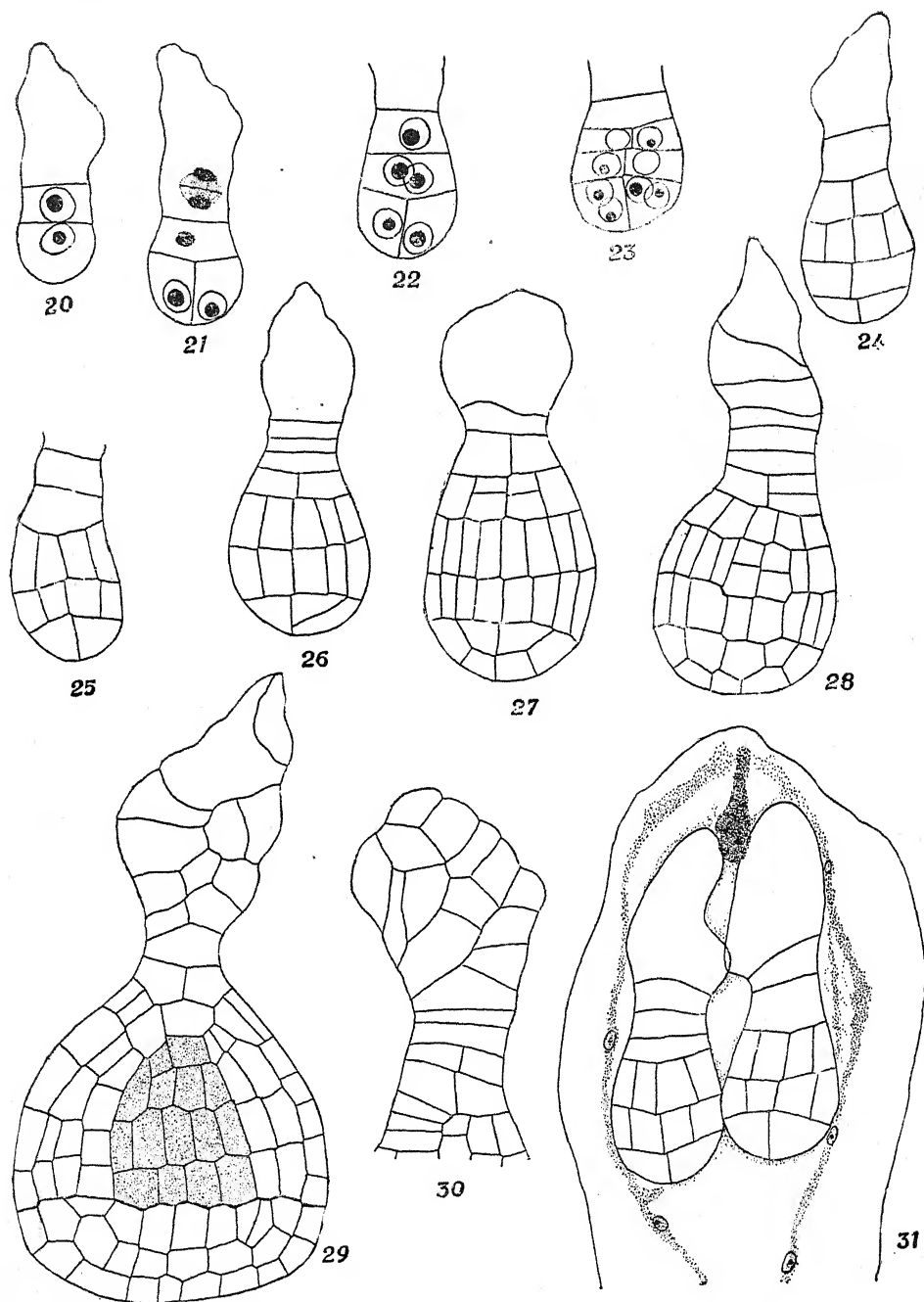
Figs. 20-31. *Boerhaavia repanda*. Figs. 20-29, various stages in the development of the embryo. The dotted part in fig. 29 represents plerome. Fig. 30 shows the structure of the suspensor. Fig. 31 represents micropylar part of an embryo-sac showing two embryos equally developed. A degenerating pollen-tube is seen at the top in between the two embryos. For further explanation see text. ✕ 450.

part of the embryo of *Boerhaavia diffusa*. Procambial tissue, similar to that described by Woodcock (1929) in *Mirabilis Jalapa,* is developed in the embryo of both the species of *Boerhaavia.*

Starch is deposited more or less uniformly in the different parts of the embryo, except in the apical part of the radicle. It is less abundant in this region, and at the extreme apex is not found at all. The starch grains in the procambial tissue are smaller in size and less abundant than in other layers. The deposition of these grains takes place rather late in the development of the embryo.

Polyembryony

An example of polyembryony has been observed in *Boerhaavia repanda.* This is shown in Fig. 31. The accessory embryo is seen to develop from a synergid.

There are two conditions, which seem to be responsible for this kind of development, the occurrence of egg-like synergids and the penetration of accessory pollen-tubes into an embryo-sac. During the course of investigations going on in this department on the embryology of the flowering plants, both these conditions have been noted quite frequently in several families of the Centrospermales. It could, therefore, be anticipated that in some cases the simultaneous presence of these characters would lead to the development of polyembryony.

In the Nyctaginaceae, the presence of accessory pollen-tubes has been recorded by Maheshwari (1929) in *Boerhaavia diffusa,* and the writer has also observed the presence of such tubes in *Boerhaavia repanda.* Two pollen-tubes were also seen penetrating the ovule showing polyembryony. The presence of egg-like synergids has been seen by the writer in *Boerhaavia diffusa.* In *Boerhaavia repanda,* the material of pre-fertilisation stages has not been examined, but as the two species are very similar in all their embryological features, egg-like synergids are likely to occur in this species as well. The vacuolation of the basal cells of both the embryos from the poly-embryonous ovule is seen to be egg-like. It can, therefore, be concluded that the polyembryony in *Boerhaavia repanda* has most probably developed from the fertilisation of an egg-like synergid by a male gamete from an accessory pollen-tube in addition to normal fertilisation.

In the family Nyctaginaceae, no other instance of polyembryony has been recorded so far. Johri (1936) has reported recently a parallel case of polyembryony in *Sagittaria graminea,* and more examples of a similar nature are cited by Dahlgren (1927).

Endosperm and Perisperm

The primary endosperm nucleus divides before the egg (Fig. 2). By its repeated division it forms a large number of nuclei, which are scattered in the protoplasmic lining of the embryo-sac. The endosperm becomes cellular in the micropylar region forming a cap
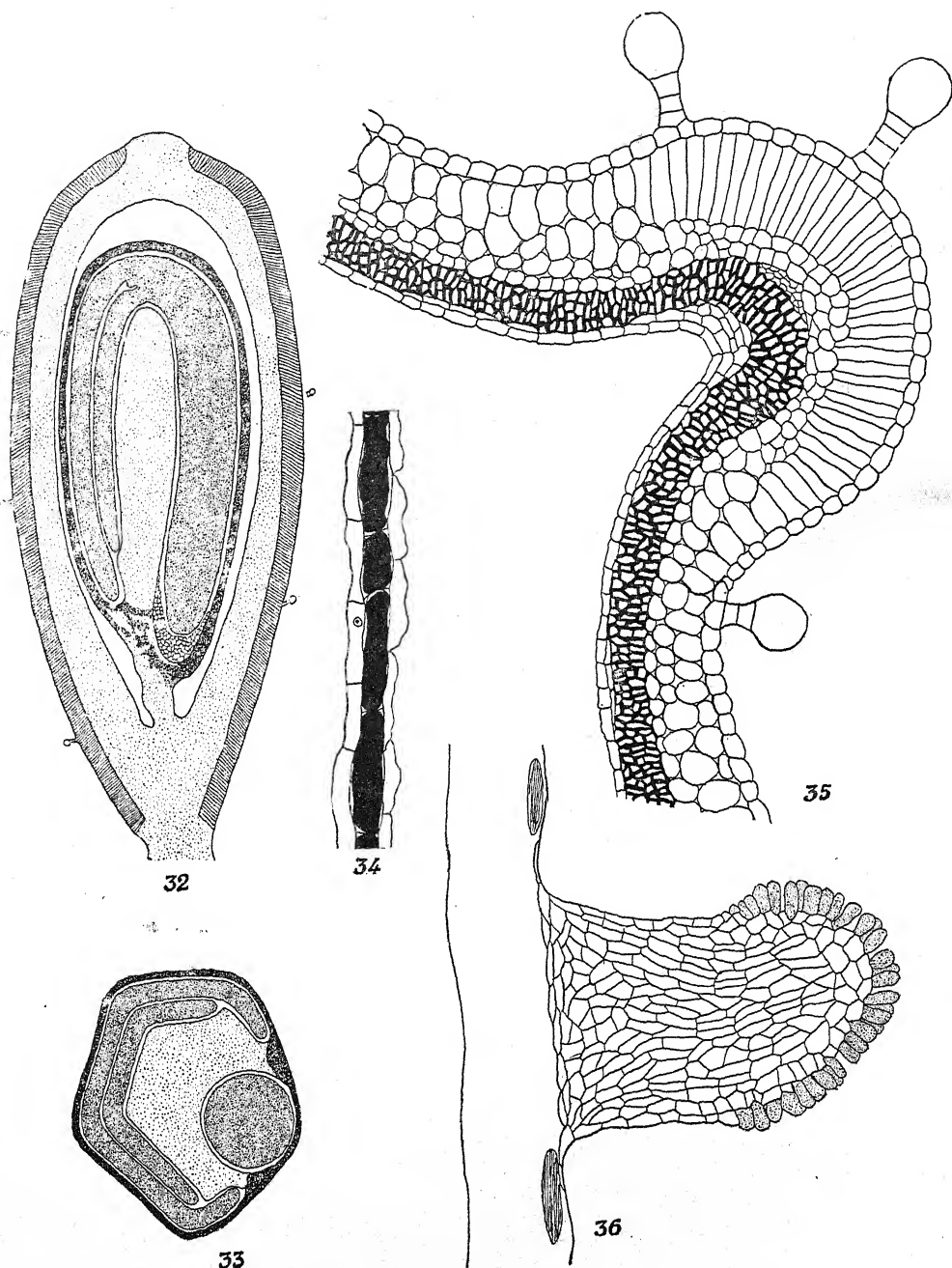
Figs. 32-36. *Boerhaavia diffusa*. Fig. 32, a longitudinal section of the fruit along with the perianth. It shows a well-developed embryo, endosperm and perisperm. Fig. 33, transverse section of the fruit showing two cotyledons, radicle and perisperm. Fig. 34, a part of the transverse section of the testa and pericarp fused together. Fig. 35, a part of the transverse section of the perianth. Fig. 36, a longitudinal section of a gland from the perianth of *Boerhaavia repanda*. Areas marked with lines represent cells containing raphides. Fig. 32 has been reconstructed from a number of adjacent sections.

of cells over the apex of the radicle (Fig. 32). The larger part of it is absorbed in the free nuclear condition by the developing embryo, as described by Woodcock (1929) in *Mirabilis Jalapa*. The endosperm development in the Nyctaginaceae thus corresponds to the *Melandrium*-type of Rócen (1927).

The central part of the nucellus is left in the mature seed as perisperm in between the cotyledons and the radicle (Figs. 32 & 33). Starch grains are abundantly deposited in this region and serve as the reserve food for the embryo.

Pericarp and Testa

The ovary wall is many cells thick to begin with, but during the development of the embryo the inner layers are crushed and only the outermost layer persists in the mature fruit. This represents the pericarp. It consists of empty cells and fuses with the testa of the mature seed as in the caryopsis of the grasses.

The testa in the fully developed seeds consists of three to four layers of cells in *Boerhaavia repanda* and two to three layers of cells in *Boerhaavia diffusa*. In the former plant the cells of the testa are empty and there is no deposition of any grains in their cells. The testa in *Boerhaavia diffusa* consists of two parts (Fig. 34). The inner part is of one or two layers of empty cells. The outer part consists of a single layer of cells, in which some grains probably of the nature of aleurone grains are deposited. These grains in the beginning are separate from each other, but ultimately they unite together and form a mass of brownish yellow substance, which occupies whole of the cell (Fig. 34). The deposition of the grains extends to the inner layers along the margins of the outer cotyledon. Some similar grains are also deposited in the region of the funicle (Fig. 32).

Structure of the Perianth

As in the Nyctaginaceae in general, the lower part of the perianth persists as an envelope for the fruit (Fig. 32). The structure of it differs in the two species of *Boerhaavia*. In *Boerhaavia diffusa,* it has five ribs and an equal number of furrows alternating with the ribs. On both sides it is covered by epidermal layers (Fig. 35). The outer epidermis bears numerous glandular hairs both in the region of the ribs and furrows (Fig. 35). Each gland has a stalk of three to five cells and a one-celled head, as described by Maheshwari (1929). Below the external epidermis under the ribs there is a layer of palisade-like sclerenchymatous cells, followed by a few layers of parenchyma cells. In the region of the furrows corresponding to these tissues is found the spongy mesophyll. Next in both the regions of ribs and furrows there is seen a continuous band of fibrous sclerenchymatous cells, followed by one or two layers of parenchymatous cells and the inner epidermis. There are five

main vascular bundles one below each rib, as described by Joshi and Rao (1934). Aleurone grains similar to those in the testa are deposited in the outer and inner epidermis and one or two layers of parenchyma on either side of the sclerenchymatous fibres. Similar grains are also commonly observed in the glandular hairs.

The perianth of *Boerhaavia repanda* has ten ribs and as many furrows. The outer surface of it is covered by large glands, which are developed on the ribs only. The structure of one such gland is sketched in figure 36. It consists of a large multicellular stalk carrying numerous glandular cells at its apex. The latter (dotted in the figure) take darker stain than the rest. They are radially elongated and have a nucleus at the base.

The wall of the perianth in *Boerhaavia repanda* consists mostly of parenchyma cells, in which ten columns of sclerenchymatous cells are developed below the ten ridges. There are ten vascular bundles, one below each rib, as noted by Joshi and Rao (1934). As in *Boerhaavia diffusa* aleurone grains are deposited in some cells of the perianth.

Raphides of calcium oxalate are abundantly deposited in both the species of *Boerhaavia* in the perianth.

Discussion

Comparison of the embryo development in the Nyctaginaceae with that of other families of Centrospermales like Caryophyllaceae, Amarantaceae, Chenopodiaceae and Molluginaceae shows that it resembles the latter families in the fact that four or occasionally five cells take part in the development of the embryo. It, however, differs in four important points. In the Nyctaginaceae after the first division of the fertilised egg, subsequent transverse divisions, up to a certain stage at least, take place in the basal cell only. This feature is in direct contrast with what is seen in the Caryophyllaceae, where the basal cell never divides and the rest of the proembryo develops only from divisions in the apical cell of the two-celled proembryo. The Chenopodiaceae occupies an intermediate position. Here both the cells, resulting from the first transverse division of the fertilised egg take part in the development of the proembryo.

The four apical cells, that form the embryo in the Nyctaginaceae, differentiate gradually unlike other Centrospermales. By the time the apical cell has divided longitudinally one or two cells out of these four are not even formed. They are cut off later by the basal cell. In other families of the Centrospermales the cells that form the embryo are all differentiated before any longitudinal division takes place in the proembryo.

The sequence, in which the longitudinal divisions appear in the proembryo in the Nyctaginaceae is quite different from what is seen in the Caryophyllaceae, Chenopodiaceae, Amarantaceae and

Molluginaceae. In the latter families the first longitudinal division takes place in the second, third or fourth cell from the apex, and then extends towards the apical cell. In the Nyctaginaceae the first longitudinal division is seen in the apical cell, next in the penultimate cell and finally in the third cell.

The development of the three histogenic layers in the Nyctaginaceae is very peculiar. The periblem originates from the dermatogen and is not a sister layer of the plerome as in other flowering plants.

Summary

The paper deals with the embryo and seed development in *Boerhaavia diffusa* and *Boerhaavia repanda*.

The first division of the egg is by a transverse wall to form a basal and apical cell. The former then divides repeatedly to make the embryo five cells long. Simultaneously with these transverse divisions progressing in the basal cell longitudinal walls begin to appear in the proembryo. The first appears in the apical cell, next in the penultimate cell and finally in the third cell from the apex. These three cells along with a fourth cell adjacent to the third give rise to the embryo proper. The apical cell gives rise to the plumule and the cotyledons. The second and third form the hypocotyl and the greater part of the radicle, while the fourth cell is the hypophysis and completes the root apex.

The differentiation of the three histogenic layers is very peculiar. The periblem is cut off from the dermatogen and is not a sister layer of the plerome as in other angiosperms.

The suspensor develops from the micropylar cell of the proembryo, after it has cut off the hypophysis cell. It is long and slender in *Boerhaavia diffusa* and short and massive in *Boerhaavia repanda*.

A case of polyembryony resulting from the development of an extra embryo from a synergid has been observed in *Boerhaavia repanda*.

The endosperm becomes cellular only in the micropylar region of the embryo-sac.

The testa and pericarp fuse together in the mature seed.

Other features of the seed are of the usual Centrospermalean type. The structure of the perianth is described in the body of the paper.

4

Literature Cited

BHARGAVA, H. R. (1932).—Contribution to the morphology of *Boerhaavia repanda. Jour. Ind. Bot. Soc.,* 11: 303-26.

DAHLGREN, K. V. O. (1927).—Die Befruchtungserscheinungen der Angiospermen. Eine monographische Übersicht. *Hereditas,* 10: 169-229.

GUEGUEN, F. (1901).—Anatomie comparée du tissue conducteur du style et du stigmate des Phanérogames. Paris.

JOHRI, B. M. (1936).—Studies in the family Alismaceae. IV. *Alisma Plantago* L., *Alisma Plantago-aquatica* L. and *Sagittaria graminea* Mich., *Proc. Ind. Acad. Sci., B,* 4: 128-38.

JOSHI, A. C. and RAO, V. S. (1934).—Vascular anatomy of the flower of four Nyctaginaceae. *Jour. Ind. Bot. Soc.,* 13: 169-86.

JOSHI, A. C. and RAO, V. R. (1936).—The embryology of *Gisekia pharnaceoides* Linn. *Proc. Ind. Acad. Sci., B,* 3: 71-92.

JOSHI, A.C. and KAJALE, L. B. (1937).—Fertilisation and seed development in Amarantaceae. *Proc. Ind. Acad. Sci., B,* 5: 91-100.

KAJALE, L. B. (1936).—Embryo development in *Boerhaavia diffusa* Linn. *Curr. Sci.,* 4: 743.

————— (1937).—A case of polyembryony in the Nyctaginaceae. *Curr. Sci.,* 5: 429.

————— (1937).—Embryology of *Achyranthes aspera* Linn., *Pro. Ind. Acad. Sci., B,* 5: 195-205.

MAHESHWARI, P. (1929)—Contributions to the morphology of *Boerhaavia diffusa* (1) *Jour. Ind. Bot. Soc.,* 8: 219-34.

ROCEN, T. (1927).—Zur embryologie der Centrospermen. Diss. Uppsala.

SOUEGES, R. (1920).—Developpement de L'embryon chez le *Chenopodium Bonus-Henricus* L. *Bull. Soc. Bot. France,* 67: 233-57.

————— (1924).—Developpement de L'embryon chez le *Sagina procumbens. Bull. Soc. Bot. France,* 24: 590-614.

WOODCOCK, E. F. (1929).—Seed studies in Nyctaginaceae. *Papers Mich. Acad. Sci. Arts and Letters,* 9: 495-502.

THE GROWTH OF RICE SEEDLINGS IN SALT SOLUTIONS OF DIFFERENT H-ION CONCENTRATIONS

BY

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In the recent work on the intake of nitrogen by the rice plant Dastur and Malkani (1933) carried out different types of water culture experiments and they have shown by chemical analysis of the culture solutions that the ammoniacal nitrogen absorption by plants is high in the early stages and low in the latter stages of growth while the absorption of the nitrate nitrogen follows the reverse order. They have also shown by using different ammonium salts that the order for the absorption of ammonia from the respective salts was sulphate, phosphate, nitrate and chloride in the early stages of growth, while in the case of nitrate salts the order of absorption of nitrate ions from the respective salts were in the order ammonium, potassium, magnesium, calcium and sodium. Greatest absorption of ammoniacal and nitrate nitrogen occurred from ammonium sulphate and ammonium nitrate respectively.

Though the absorption of nitrogen from different ammonium salts and nitrate may depend on the kind of the salt used, it does not follow necessarily that the growth of plants kept in these salts was affected in the same order as the absorption of nitrogen from these solutions. These authors have not measured side by side the growth of the rice seedlings kept in the different solutions of ammonium and nitrate salts. Whether greater absorption of ammonium or nitrate ions from these salts, viz., ammonium sulphate and potassium nitrate, is accompanied by greater growth of the Rice seedlings kept in these solutions is not determined. If this is so, the growth made by the seedlings would be less and less in the salts of ammonia in the order sulphate, phosphate, nitrate and chloride. Similarly the growth made by the seedlings in the solutions of different nitrates would be less and less in the order ammonium, magnesium, calcium and potassium nitrates during the early stages of rice seedlings.

It was, therefore, undertaken to measure the growth of the seedlings kept for a definite period in salts containing ammonia and nitrate. It was found useful not to restrict the scope of experiments to single salt solutions but also to use different combinations of these salts. The object of this investigation is to find out the solution in which the growth of the rice seedlings is at its maximum.

In order to carry on this investigation, different factors affecting the growth of seedlings in culture solutions must be taken into account so as to obtain true relationship between the growth of the seedlings and the salt solutions used. Firstly, the importance of the acidity and alkalinity of the medium on the growth of plants is very well known, and important data on the relation between plant and the alkalinity and acidity of the external media are already collected on different plants by various workers—*i.e.* Hoagland (1918), Duggar (1920), McCall and Haag (1921) and many others.

Jacobson (1925) grew rice plants in four different nutrient solutions and found that the pH value decreased from $5 \cdot 0$ to about $3 \cdot 5$ during the first 24 hours and it remained practically constant for 3 days. It may be due to the absorption of the basic ions in a greater proportion than that of the acidic ions of the salts or it may as well be due to the excretion of carbon dioxide by the plant-roots. In India, Mitra and Phukan (1929) determined the effect of different pH values of nutrient solutions on the growth of the roots of rice seedlings one month old. They used the length of the roots as a measure of growth and found that the highest root development was obtained at a concentration of $0 \cdot 03\%$ of NaOH, *i.e.*, at pH $7 \cdot 9$ and that pH $3 \cdot 9$ was distinctly toxic.

From the review of the previous work given in the above para, it appears that two important points must be taken into consideration in the arrangement of the experiments. The pH of each salt solution must be so controlled by the addition of weak alkali or weak acid as to obtain a wide range of pH values with a graduation of $0 \cdot 2$; secondly the pH of the solution should be kept constant by the addition of weak acid or alkali as the case may be every twenty-four hours during the period of experimentation. This is necessary as the plants affect the reaction of their external media. Amongst those who have worked on the subject Veitch (1902), Breazeale and Le Clerk (1912), Jones and Shive (1922), Hoagland (1918), Conner and Sears (1922), and Olsen (1923) may be mentioned. Some workers found that the direction of the pH changed towards neutrality if the original pH was towards the acidic side while others found that the direction of the change depended on the plant used. Arrhenius (1922) has studied the effect of the rice plant on the pH of the nutrient solutions. In all cases the rice plant brought the pH of all nutrient solutions with different initial values to $6 \cdot 2$.

The second important factor to be taken into consideration is the total osmotic values of the nutrient solutions used. The osmotic

values of the nutrient solutions used should be approximately the same, otherwise the comparison of growth made by the rice plants in different solutions may not be strictly valid. For this reason iso-osmotic solutions of different salts should be used. The salt solutions should be periodically renewed, preferably twice a week, otherwise the concentration-changes brought about in the solutions may effect adversely the growth of rice seedlings.

Methods Adopted

The H-ion concentration of the cultures and various salt solutions used were determined by the colorimetric method given by Clark (1920). The results of pH obtained by this method were also verified by the quinhydrone electrode method in some cases.

In order to obtain the desired pH value of a salt solution, weak alkali or weak acid as required was used. The acid used was $0 \cdot 2$ Normal phosphoric acid and for the alkali N/40 sodium bicarbonate was used.

Rice seeds of the Columba variety 42 were soaked in water for 24 hours and allowed to germinate in saw dust. Watering was done twice a day, once in the morning and once in the evening. When they were 14 days old they were taken out of the saw dust and washed thoroughly first with tap water and then with distilled water. Healthy seedlings with equally developed roots were selected from this lot. The length of the roots taken was generally 4 inches. 480 such seedlings were selected for each series.

Cylindrical jars of about 1,500 c.c. capacity are chosen, and in each jar 30 seedlings are kept to grow. The jars are fitted with wooden covers bearing six holes, each hole carrying 5 seedlings. The inside of the jars was completely paraffined so as to prevent any change in the pH value of the solutions due to the solubility of glass in water.

The culture solution used was that given by Knops as modified by Tottingham.

It was first undertaken to determine the effect of the omission of iron from the normal culture solution on the growth made by the rice seedlings as there is a great deal of difference of opinion on the availability of iron to the rice plant. It has been maintained by Gile and Carrero (1920) and Willis and Carrero (1923) that nitrates are less valuable as a source of nitrogen than ammonium salts since the former cause unfavourable soil reaction and unavailability of iron, though this view has been disputed by Metzger and Janssen (1928).

It was, therefore, first undertaken to study the effect on the growth of Rice seedlings of (*i*) omission of iron, (*ii*) addition of ferrous sulphate and ferric phosphate separately and (*iii*) replacing

pctassium nitrate by ammonium sulphate in the culture solutions. The pH of each of these solutions was kept varied from 3·6 to 7·0 with a graduation of 0·2 pH except in the first case where the graduation is 0·6 pH.

Four series of experiments containing (*i*) culture with $FeSO_4$, (*ii*) culture with $FePO_4$, (*iii*) culture without Fe and (*iv*) culture with $(NH_4)_2SO_4$ instead of KNO_3 were started. The experiments were carried in triplicate. The 14 days old seedlings germinated in saw dust were kept in the culture jars and they were allowed to grow for a fortnight in all cases. The results are given in Table I, and Figure 1.

TABLE I

Weights of roots and leaves in mgs. of Rice Seedlings
Average of 3 sets of experiments

pH	Culture with $FePO_4$	Culture with $FeSO_4$	Culture without Fe	Culture with $(NH_4)_2SO_4$ in place of KNO_3
3·6	233·3	233·6	233·2	233·4
4·2	238·2	234·0	233·1	233·6
4·4	239·6	235·0	233·7	234·7
4 6	239·6	236·0	234 8	235·3
4·8	240·0	236·1	235·0	235·0
5·0	241·7	235·6	235·2	236·8
5·2	242·5	236·7	236·3	238·0
5 4	246·3	238·0	237·0	237·4
5·6	247·4	239·0	238·0	239·4
5·8	248·6	240·0	239·0	241·4
6·0	249·4	243·6	240 0	242·0
6·2	250·2	244·3	244·0	242·8
6·4	251 8	245·0	245·0	244 0
6·6	252·5	245 7	247·0	244·6
6·8	260·0	246·5	249·0	245·6
7·0	261·3	246·0	250·0	245·5
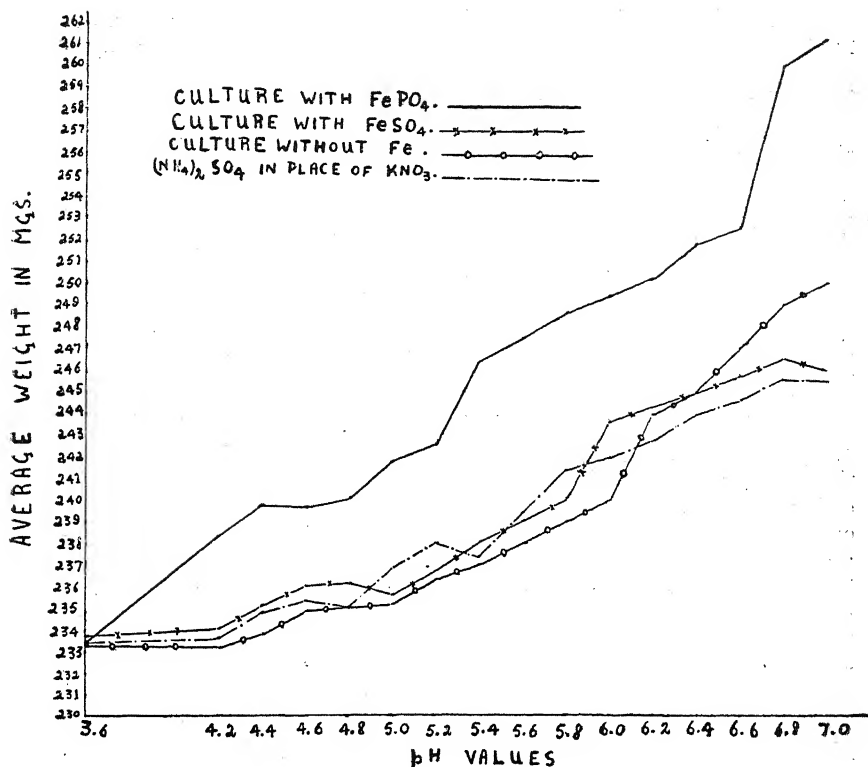
Average weights of rice seedlings grown in different culture solutions.

In order to determine the solution in which the highest growth of the seedlings takes place, it was necessary to analyse statistically the data by Fisher's method of Analysis of Variance. The total variance is to be divided into that due to different pH, that due to different treatments and the residual variance, which in the present case is the variance due to the interaction of pH and treatments. Results of differences between the mean yields are given in Table II.

TABLE II
Differences between the mean yields

	Culture with $FePO_4$	Culture without $FePO_4$	Culture with $FeSO_4$	Culture with $(NH_4)^2SO^4$
Culture with $FePO_4$	—	−59·4	−60·2	−63·0
Culture without $FePO_4$	+59·4	—	− 0·8	−3
Culture with $FeSO_4$	+60·2	0·8	—	− ·8
Culture with $(NH_4)_2SO_4$	+63·0	3·6	2·8	—

The culture with $FePO_4$ is highly superior to all the other three treatments, the differences between which are statistically non-significant. The above conclusion is evident from the examination of Table I and the graphs given in Fig. 1. It can also be seen that the pH of each culture solution has great effect on the growth of the rice seedlings irrespective of the salts used. The rice seedlings show very little growth in very acidic solutions. The dry weights of the seedlings are very low in the solutions of pH 3·6—5·0. The growth is the highest in solutions of very low acidity with pH between 6·6 and 7·0.

Replacement of potassium nitrate by ammonium sulphate in the culture solution shows no beneficial effect on the growth of the seedlings.

In the second year of this investigation it was undertaken to study the effect of nitrates and ammonium salts on the growth of the rice seedlings. In these experiments the procedure adopted differed in two important aspects. In the first case the pH of each culture solution was maintained the same by the addition of phosphoric acid or sodium bicarbonate as the case may be every 24 hours. The procedure of bringing the pH of a solution to its initial value every 24 hours is described above in the methods. The second important departure was the renewal of culture solutions every week. This was necessary in order to maintain the original ionic concentrations of the salts used. Period of one week is selected on account of the findings of Dastur and Malkani (1933) that an equilibrium between the internal concentration and external concentration of an ion like the ammonium ion is reached when the rice plants are kept for a week in a solution of salts.

In the first place three nitrate salts and three ammonium salts were taken and the solution of these salts were individually used for determining the effect on the growth of the rice seedlings. The range of pH values used in each case was the same as before and the experiments were made in triplicate series. The salts used were nitrates of potassium, magnesium and sodium and the ammonium salts used were sulphate, phosphate and chloride.

The results obtained are very interesting. The growth of the rice seedlings is greater in the solutions of high pH than in the solution with low pH in the case of all the salts. In the case of potassium the dry weight of the seedlings increases in solutions of pH above 5·0; it means that higher acidity in potassium nitrate solutions retard growth. The same is true of the dry weights of the rice seedlings in the ammonium sulphate solutions. In the case of ammonium phosphate solution marked increase in dry weight occurs in solutions of pH values lower than in the preceding two cases, and beyond pH 6·6 the dry weights of the seedlings show a definite decline. Very little growth is made in ammonium chloride

solutions while it is medium in solutions of nitrates of magnesium and sodium. Thus the most favourable pH in the acidic range is near $7·0$ except in the case of ammonium phosphate where the most favourable range of pH for growth lies between $6·2$ to $6·6$. It appears that in the solutions of different salts with a low pH value, the latter becomes a limiting factor in the growth of the seedlings irrespective of the nature of the salt used. The differential effects on growth of the seedlings of different salts become visible as the pH values of the solutions rise from about $5·0$ onwards. Then the different salts used stand in the order $(NH_4)_2SO_4=(NH_4)_3PO_4 > KNO_3 > Mg(No_3)_2 > NH_4Cl=NaNO_3$ so far the growth of the seedlings can be judged from the results in Table III and Fig. 2.
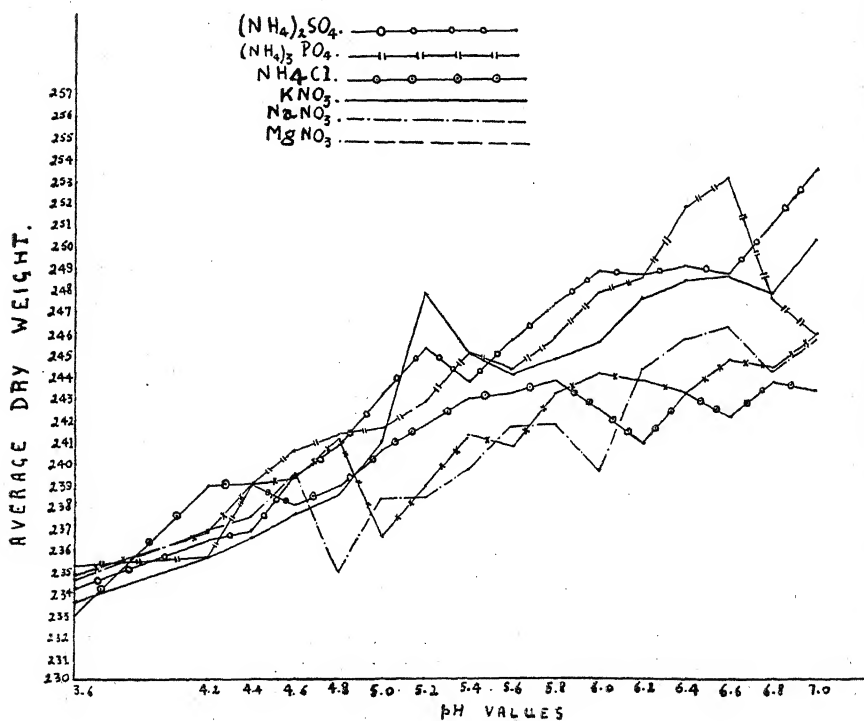

FIG. 2.

Mean values of the dry weights of the rice seedlings in solutions of nitrates and ammonium salts.

TABLE III.

Mean values of the dry weights of the rice seedlings in solutions of nitrate and ammonium salts.

pH	KNO_3	$NaNO_3$	$Mg(NO_3)_2$	$(NH_4)_2SO_4$	$(NH_4)_3PO_4$	NH_4Cl
3·6	233·6	234·6	234·3	234·2	235·3	233·0
4·2	235·6	236·9	236·5	236·4	235·6	238·9
4·4	236·5	237·5	239·8	236·8	239·0	239·0
4·6	237·6	239·5	239·3	239·4	240·5	238·0
4·8	238·5	235·0	241·1	240·8	241·3	238·9
5·0	240·9	238·3	236·6	243·1	241·6	240·6
5·2	247·8	238·4	238·9	245·3	242·8	241·7
5·4	245·1	239·7	241·3	243·8	245·1	243·0
5·6	244·1	241·7	240·8	245 7	244·3	243·2
5·8	244·8	241·8	243·2	247·4	245·8	243·8
6·0	245·6	239·7	244·1	248·8	247·9	242·5
6·2	247·6	244·3	243 8	248·7	248·6	240·9
6·4	248·4	245·7	243·2	249·1	251·8	243·2
6·6	248·6	246·3	244·7	248·7	253·1	242·1
6·8	247·8	244·2	244·4	251·0	247·6	243·7
7·0	250·2	245·7	245·9	253·5	245·9	243·3

These results of the dry weights of the seedlings are statistically examined according to the method described, in order to determine the salt solution in which highest growth has occurred taking all the solutions of different pH values considered together. The difference between the mean yields in various treatments (*i.e.,* salt solutions) are given in Table IV in which any two treatments may be compared and their relative superiority determined. It will be seen that ammonium sulphate is superior to all other treatments. It is better than ammonium phosphate but the differences between the two are not significant. Sodium and magnesium nitrates and ammonium chloride have proved to be much inferior treatments. Thus the results conclusively confirm the superiority of ammonium

sulphate over potassium nitrate for the growth of rice seedlings in the early stages of growth.

TABLE IV

Table of difference between the mean yields.

	KNO_3	$NaNO_3$	$Mg(NO_3)_2$	$(NH_4)_2SO_4$	$(NH_4)_3SO_4$	NH_4Cl_2
KNO_3	—	−23·55	−22·05	+14·65	+ 8·50	−20·85
$NANO_3$	+23·55	—	+ 1·50	+38·20	+31·70	+ 2·70
$Mg(NO_3)_2$	−22·50	− 1·50	—	+36·70	+30·20	+ 1·20
$(NH_4)_2SO_4$	−14·65	−38·20	−36·70	—	− 6·50	−35·50
$(NH_4)_3PO_4$	− 8·50	−31·70	−30·26	+6·50	—	−29·00
NH_4Cl	+20·85	− 2·70	− 1·20	+35·50	+29·00	—

Double lines indicate significance at 1% level.
Single lines indicate significance at 5% level.

It was also undertaken to use a two-salt solution containing three or four different ions in different combinations to study the effect of the different ions when used singly or in presence of other ions. It would also help in determining the effect of the nitrate ions in presence of ammonium salts and *vice versa,* on the growth of the plants. It is not attempted to use all the combinations possible as the number of experiments becomes too large to manage but the following combinations have been used. Ammonium phosphate is combined separately with nitrates of sodium and magnesium. Ammonium sulphate is combined with calcium or sodium nitrate and ammonium chloride is used in combination with calcium or magnesium nitrate. In one series of experiments calcium and magnesium salts are combined to study their effect on the plant while in another case two sulphate salts are combined for the same reason. Side by side, it was found necessary to start a series of experiments with the culture solutions containing all the essential ions.

The results of the dry weights of the rice seedlings grown in different solutions containing mixtures of two different salts and in normal culture solutions are given in Table V.

TABLE V

Mean values of the dry weights of the Rice Seedlings in Culture of two salt solutions.

pH	Culture	(NH₄)₃PO₄ NaNO₃	(NH₄)₃PO₄ + Mg(NO₃)₂	(NH₄)₃PO₄ NH₄Cl	NH₄Cl MaNO₃	NH₄Cl Mg(NO₃)₂	(NH₄)₂SO₄ Ca(NO₃)₂	(NH₄)₂SO₄ + MgSO₄	MgSO₄ Ca(NO₃)₂
3·6	234·1	235·7	236·3	235·4	234·6	235·5	233.4	233·1	233·2
4·2	239·3	238·0	240·0	238 7	239·5	239·0	235·1	235·4	236·4
4·4	241·1	236·0	240·5	239·9	239·7	239·1	236·1	235·5	234·9
4·6	242·8	238·0	240 6	240·8	240·6	239·0	236·7	231·7	237·2
4 8	242·9	238·0	239·1	239·4	239·4	241·0	236·7	237·6	235·6
5·0	244·3	236·8	240·4	239·2	240·4	239·0	230·0	237·9	238·0
5·2	245·6	239·1	239·1	242·3	241·3	240·8	238·6	236·7	239·2
5·4	247·6	242·5	240·4	242·7	243·1	239·3	238·7	237.4	238·5
5·6	248·7	243·8	242.7	243·7	243·7	240·9	239·7·	238·1	239·1
5·8	250·4	244·6	243·7	245 3	244·5	241·1	240·0	240·2	240·1
6·0	252·7	244·6	245·8	246 3	245·2	242·6	240·1	233·9	240·1
6·2	253·5	245·7	246·9	247·6	245·9	244·0	241·9	240·3	240·5
6·4	254·8	245·9	247·6	248·6	246·6	242·5	243·6	238·3	241·8
6 6	255·7	247·8	248·9	246 7	244·4	242·8	245·4	241·9	242·1
6·8	256·2	244·9	247·6	246·2	243·2	239·0	245·3	238·3	243 5
7·0	257·1	246·1	245·4	246·8	244·0	239·2	245·9	240·3	244·9

In the culture solution the dry weights of the seedlings increase steadily as the pH value rises from 4·2 to 7·0 thus agreeing with the results discussed above. Growth of the seedling is retarded in acidic media. The same remarks can be applied to the results of the different mixtures containing ammonium phosphate. In case of ammonium phosphate solution, the best growth is made in solutions of pH 6·2—6·8 (Table V). In the case of ammonium chloride solution with sodium nitrate or magnesium nitrate the pH from 5·6 to 6·6 appears to be the most favourable for growth (Table V). In case of mixture of ammonium sulphate and calcium nitrate the acidic media from pH 3·6 to 5·0 are definitely detrimental to the growth of the seedlings and the best growth is made in the solution of pH 6·6 to 7·0. It is difficult to say the range of pH favourable to growth in case of the mixture of ammonium sulphate and magnesium sulphate as very little increase in dry weights are noticed in all cases. In case of the mixture of calcium nitrate and magnesium sulphate, highest dry weights are obtained near the pH 6·8 to 7·0. In the case of the mixtures of these two salts highly acidic media are injurious to the growth of the rice seedlings. As the results stand in Table V, culture solution appears to be the best solution for the growth of the rice seedlings. The mixtures of the ammonium phosphate with the three different salts are the next best, but the growth of the rice seedlings is not as vigorous as in the case of pure ammonium phosphate solution. The presence of nitrates of sodium and magnesium and ammonium chloride shows a depressing effect (Table V). The mixture of ammonium chloride with sodium nitrate was found better than the mixture of ammonium chloride with magnesium nitrate. In the latter case there is very little growth made by the seedlings. The mixture of ammonium sulphate with calcium nitrate is found less beneficial to the growth of the rice seedlings than ammonium sulphate alone, while the mixture of ammonium sulphate with magnesium sulphate was found definitely bad. The presence of magnesium is found harmful to the growth of the rice seedlings except in the presence of ammonium phosphate.

The statistical examination of the results has been carried out as before and the results of the differences in the mean values of the yield are presented in Table VI. It will be seen that culture is superior to all treatments at 1% level of significance. Mixtures of ammonium phosphate with different salts are the next best of which the mixture of ammonium phosphate and ammonium chloride appears better than the mixture of ammonium phosphate with two nitrates, though the differences between them are not significant. The mixtures of ammonium sulphate with magnesium sulphate and with calcium nitrate have proved to be the most inferior. The same remarks apply more or less to the rest of the treatments.

TABLE VI.

Table of differences between the mean yields.

Culture	$(NH_4)_3PO_4 + N\,NO_3$	$(NH_4)_3PO_4 + Mg(NO_3)_2$	$(NH_4)_3PO_4 + NH_4Cl$	$NH_4Cl + NaNO_3$	$NH_4Cl + Mg(NO_3)_2$	$(NH_4)_2SO_4 + Ca(NO_3)_2$	$(NH_4)_2SO_4 + MgSO_4$	$MgSO_4 + Ca(NO_3)_2$
Culture	− 57·50	− 51·65	− 48·15	− 56·65	− 76·35	− 88·50	−103·25	− 89·05
$(NH_4)_3PO_4 + NaNO_3$	−	+ 5·86	+ 9·35	+ 0·85	− 18·75	− 31·00	− 45·75	− 31·55
$(NH_4)_3PO_4 + Mg(NO_3)_2$	− 5·35	−	− 3·50	− 5·00	− 24·60	− 36·85	− 51·60	− 37·40
$(NH_4)_3PO_4 + NH_4Cl$	− 9·35	− 3·50	−	− 8·50	− 28·10	− 40·35	− 55·10	− 40·90
$NH_4Cl + NaNO_3$	− 0·85	− 5·00	− 8·50	−	− 19·60	− 31·85	− 46·60	− 32·40
$NH_4Cl + Mg(NO_3)_2$	+ 18·75	+ 24·60	+ 28·10	+ 19·00	−	− 12·25	− 27·00	− 12·80
$(NH_4)_2SO_4 + Ca(NO_3)_2$	+ 31·00	+ 36·85	+ 40·90	+ 31·85	+ 12·25	−	− 14·75	− 0·55
$(NH_4)_2SO_4 + MgSO_4$	+ 45·75	+ 51·60	+ 55·10	+ 46·60	+ 27·00	+ 14·75	−	+ 14·20
$MgSO_4 + Ca(NO_3)_2$	+ 31·55	+ 37·50	+ 4·35	+ 32·40	+ 12·88	+ 0·55	− 14·20	−

Double lines indicate superiority at 1% level of significance.
Single line indicates superiority at 5% level of significance.

Summary

In their studies on the intake of nitrogen Dastur and Malkani (1933) have shown that rice seedlings absorb more of ammoniacal nitrogen than the nitrate nitrogen from their respective salts. They have also determined the order of absorption of the ammonium and nitrate ions from their respective ammonium salts and nitrates and they have shown that the ammonium salts stood in the order sulphate, phosphate, nitrate and chloride as far as the absorption of ammonium ion was concerned.

Similarly the nitrates stood in the order ammonium, potassium, magnesium, calcium and sodium.

In the above investigation the effect on the growth of the rice seedlings of the absorption of these ammonium and nitrate ions from different salts is not determined. It is necessary to determine if the greater absorption of the ammonium ion is accompanied by greater growth of the seedlings as compared with the growth of the seedlings kept in solutions of nitrates.

The effect of the hydrogen ion concentration of the salt solution on the growth of plants is well-known and it is therefore necessary to study the growth of rice seedlings in salt solutions of different pH values.

The pH value of each salt solution has great effect on the growth of the rice seedlings. The results show that in all cases the greatest growth occurred in the salt solutions whose pH ranged from about $6 \cdot 0$ to $7 \cdot 0$. Very little or no increase in dry weight was noticed in the salt solutions with low pH values indicating that acidity lower than $6 \cdot 0$ pH was detrimental to the growth of the seedlings.

The results of these experiments were statistically interpreted by using Fisher's method of analysis of variance and as a result of that the following facts emerged.

Culture with Ferric Phosphate was superior to culture with $FeSO_4$. Similarly culture with Ferric phosphate was superior to culture without iron. No difference was found between the culture with $FeSO_4$ and culture without iron. Culture with potassium nitrate as a source of nitrogen was found superior to culture with ammonium sulphate.

Amongst the single salt solutions ammonium sulphate and ammonium phosphate are found superior to potassium nitrate as the dry weights of the seedlings are higher in the former than in the latter. In the other nitrates and ammonium salt solutions the growth of the seedlings is poor.

In the two salt solutions those combinations which contain ammonium phosphate are found to be superior to other combinations.

The ordinary culture solution is found to be the best of all the solutions used.

Thus for the growth of the rice seedlings ammonium sulphate and ammonium phosphate are found to be better than potassium nitrate and the most favourable pH for the growth of the rice seedlings lies between 6·0 and 7·0, *i.e.*, towards the neutral point.

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References

1. ARRHENIUS (1922).—Soil Sci., *14*, 21–26.

*2. BREAZEALE, J. F., and LE CLERE, J. A. (1912).—U. S. Dept. Agri. Bur. Chem. Bul., 149–18.

3. CLARK, W. M. (1920).—Determination of Hydrogen ion concentration. Baltimore.

4. CONNER, S. D., and SEARS, O. H. (1922).—Soil Sci., *13*, pp. 23–33, pl. 1–4.

5. DASTUR, R. H., and MALKANI, T. J. (1933).—Ind. J. Agri. Sci., *3, 157.*

*6. DUGGER, B. M. (1920).—Ann. Mo. Bot. Gard., *7*, 1–50.

7. GILE, P. L., and CARRERO, J. O. (1920).—J. Agri. Res., *20*, 33–62.

8. HOAGLAND, D. R. (1918).—Science N. S., *48, 422–425.*

9. JACOBSON, H. G. M. (1925).—Jour. Amer. Soc. Agron., *17*, 577–583, 583–586.

10. JONES, L. H., and SHIVE, J. W. (1922).—Bot. Gaz., *73*, 391–400.

11. McCALL, A. G., and HAAG J. R. (1921).—Soil Sci., *10*, 12–69.

12. METZGER, W. H., and JANSSEN, G. (1928).—Jour. Amer. Soc. Agron., *20*, 459–476.

13. MITRA, S. K., and PHUKAN, L. N. (1929).—Agri. J. Ind., *24*, 109.

*14. OLSEN, CARSTEN (1923).—Compt. Rend. Lab. Carbsberg, *15*, 153–164.

15. VEITCH, F. P. (1902).—Jour. Amer. Chem. Soc., *24*, 1120–1128.

16. VEITCH, F. P. (1905).—U. S. Dept. Agri. Bur. Chem. Bul., *90*, pp. 183–187.

16. WILLIS, L. G., and CARRERO, J. O. (1923).—J. Agri. Res., *24*, 621–640.

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* Original not seen.

CONTRIBUTION TO THE KNOWLEDGE OF FLORA AND VEGETATION IN THE CENTRAL HIMALAYAS

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When *A. Heim* and *A. Gansser* in 1936 went on a geological expedition to the central Himalayas they also collected plants. The result of the trip, some 200 species, was given to the Botanical Museum of the University of Zürich, where the plants will be determined. They also made ample notes concerning Flora and Vegetation, chiefly in the alpine region, which allow us to form a good idea of the distribution of the Flora there. In the following we will try to sketch its relations to the great eurasiatic vegetation units.

The subalpine Forest Belt (Larch-Pinus Cembra Belt)

In the valleys of the northern Kumaon, in the frontier region towards N. W. Nepal and Tibet, the subalpine forest consists mainly of *Betula utilis* (see plate IX) ; occasionally, Conifers, *Pinus excelsa* and *Abies spectabilis* (=*A. Webbiana*) are so frequently to be found that they predominate in the aspect of the forest. In the Kali valley, between Tinkar and Garbyang, a forest of birch and white spruce with solitary pines is mentioned, below Changu an "aromatic pine forest", again above Garbyang rear Kalapani (3800 m) a pine forest, and the same between Gunji and Garbyang "along the road" and near Gunji (3175 m). Near Sepu (3490 m), a birch wood intermixed with some firs was seen on the shady side, near Rilkot "in Tola" "some fir wood", along the Kuari pass at 3500 m "firs with Rhododendron," and there also, between 3550 and 3650 m altitude a birch forest with "oak and big Rhododendron." On gravel terraces and cones of rubbish, Conifers predominate very frequently (see Pl. X). *Betula utilis* reaches enormous size. The Holy Birch near Tijang in the Valley of Lissar, at an altitude of 3300 m, has been found by Heim to have

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a diameter of 25 m in the crown, and its two main trunks measure 1,5 and 1,2m (see Pl. XI). Near the limit of the forest the trees are smaller and crooked, as in *Betula tortuosa* of the Alps.

The undergrowth of the subalpine forest consists of shrubs of Rhododendron species, such as *Rh. Anthopogon, Rh. campanulatum, Rh. lepidotum*, then *Lonicera—, Ribes—, Cotoneaster—, Rosa species,* and between them some few *Podophyltum, Allium, Cortusa Matthioli, Primula nivalis* and others.

There is no fully matured soil, as the steep slopes do not permit it. Considerable layers of raw humus are often lying on the mineral soil which is so little weathered that the humus can easily be carried away. The soil of pine woods on gravel terraces or cones too has often A—C profiles, *i.e.,* the humus lies directly on the not or only very little disintegrated mineralic ground.

The subalpine zone dominates the region between 3100 and 3900 m of altitude (near Kuti and in the Soringonga valley along the Dutuk Dhura-pass up to 4000 m), but it is well developed on the north-exposed slopes only. On the south-exposed sides of the larger valleys, the forest is scanty or even missing completely, either destroyed by men (fire places frequent) to gain pasture ground, especially near villages and caravan tracks, or due to climatic conditions, as dryness does not allow the forest to develop. Its place is taken by low shrubs of *Juniperus communis, J. Wallichiana, Cotoneaster—, Rosa—, Salix—,* and other species with a more xeric character than the society of Betula-Rhododendron. Nevertheless, mixed with these shrubs are some high herbs of subalpine character, such as *Rheum, Anemone, Geranium, Podophyltum, Epilobium latifolium, Sweertia petiolata*. In more humid places were found luxuriant meadows of herbaceous perennials; when going up from Ralam to the Dutuk Dhura-pass, Heim noticed luxuriant perennials of manured lair flora, and plains covered with fern, on the Ralam-pass high grown meadows with *Pedicularis—, Gentiana—, Geranium—* and *Orchis* species, in the dryer valley of Kuti places with *Primula—, Myosotis—, Ranunculus—, Geranium—, Corydalis—, Delphinium—, Aster* and *Anaphalis* species, *Hierochloa laxa, Polygonum affine, Anemone polyanthes, Kaschmirica,* etc. Extensive thickets of *Rosa sericea* and the less spread *R. macrophylla, Prunus* species on road sides and near villages depend on the influence of man; alpine pastures, which are found chiefly on moraine ground and gravel terraces are very rich in flowers (in July); in the valley of Kuti, *Potentilla argyrophylla* with its yellow or red flowers was surprisingly frequent. Already our actual scanty knowledge of the subalpine forest zone of the Himalayas is sufficient to show that we can very well compare it to corresponding regions in Eurasia. Most genera occur in vicarious, partly even in identical species. Such a forest belt, consisting of larch-trees, pines, and birches, forms the upper zone of all mountain forests, from Himalayas over the west—, central— and north-Chinese mountains to the subarctic Siberian plains and

to North-America, and from the South Siberian mountains to the mountains of north and central Europe. The vegetation of the inner Kumaon valleys also shows a great physiognomic resemblance to that of the central Asiatic mountains, Tianshan, Altai, and even to the dry inner alpine valleys. The north-exposed slope is covered with forest of *Pinus* spec., *Betula* resp. *Larix*, with a luxuriant undergrowth of Rhododendron; on the south-exposed side we find here and there isolated woods of these species of trees, surrounded by large bushes of Juniperus. Forests of larch, pine or birch are here limited by the forest-steppe. In the inner alpine chains, the larch—*Pinus Cembra* stage lies about 1500 m lower than in north Kumaon, between 1500 and 2400 m; in eastern Afghanistan between 3000 and 4000 m, in Hindukusch between 3000 and 4000 m, in the eastern Himalayas between 3000 and 4300 m; in east-Tibet between 3200 and 4300 m, reaches in Tsinling-shan up to 3200 m, in Wutai-shan up to 3000 m, and in Wuling-shan up to 2200 m (compare Limpricht, 1922). The richest flora of this belt is found in the old mountain center of East-Tibet, in West-China, and in south-eastern Siberia; it is poor in the north-western plains and in Europe. As in the Alps, this vegetation belt has also its southern limit in the Himalayas but here it is much better represented. Whereas in the Alps, the glacial periods have separated the flora types of the subalpine (and alpine) belt from those of lower zones in such a way that their genetic relations to these lower positions have been destroyed and are only found as relicts in island-like places, in the Himalayas (and still more in the east-asiatic mountains) the stock of species of the subalpine belt is much more genetically connected with that of the lower belts (compare Diels 1901); one has to think only of the genera *Delphinium, Thalictrum, Corydalis, Astragalus, Vaccinium, Rhododendron, Sweertia, Scrophularia, Pedicularis, Scutellaria, Prenanthes, Saussurea,* and others.

If the subalpine forest belt of the Himalayas is much better developed than in the Alps, this is because there was no destruction in the glacial period (Heim says that the glaciers did not come down to an altitude lower than of 2000 m), and it is much nearer to the floristically rich regions of the East. In both mountains, a large part of the flora of this belt has an autochthonous character.

In this subalpine belt, the cultivation by man is found as high as 3660 m in Ralam, 3490 m in Sepu, 3425 m in Milam, 3750 m in Kuti. In these fields are cultivated 6-rowed barley (even at 4300 m), potatoes, buckwheat, spinach, parsley, onions, radishes, and turnips. In Kuti there was found an area of about 1 km. sq. with two sorts of barley and two species of buckwheat. Potatoes are sown about the 10th June, and gathered in October. The cultures suffer from late frost and sudden heat. In the high villages, only a few families remain in winter, as for instance 3 out of 80 in Kuti.

The Pulsatilla—Forest-Steppe—Belt

The subalpine forest has two climatic boundaries, one thermic, which is in Kumaon at the same time the altitude limit for the birch—Rhododendron forest, and for the forest on the whole, and another which is a xeric limit. At this xeric limit there is a zone of clear forest or bush between forest and steppe in which is found an undergrowth of temperate xerophile herbs and shrubs, to a large extent bioclimatically independent of the tree-species. This forest-steppe belt is frequent in the northern part of Eurasia; it encloses in the south the eurosiberian forests of conifers, enters in Europe for reasons of plant history (displacement of Taiga in the glacial period) into the regions of angiosperm forests. The same occurs in northern East-Asia. It is found on the dry slopes of the steppe-mountains in Central-Asia, and occupies the southern slopes of the dry mountain valleys from the Alps to the North-Chinese mountains. For the Pulsatilla-forest-steppe belt are characteristic: *Pinus*—, *Juniperus*—, *Pulsatilla*—, *Astragalus*—, *Berberis*—, *Caragana*—, *Erysimum*—, *Festuca*—, *Galium* (boreale, verum)—, *Goodyera*—, *Koeleria*—, *Onosma*—, *Thesium*—, and other species.

We conclude from Heim's notes and collections that this belt is not well represented in Kumaon. There are mentioned on the south-exposed slopes of the innermost valleys from altitudes below and above those of the subalpine birch forest belt, dry shrubs, for instance in the Satopnath valley above Badrinath at 3700 m *Juniperus Wallichiana*, *J.* cf. *communis*, *Ephedra Gerardiana*, *Salix* spec., from Gunji on gravel cones up to 3900 m forming thick bushes with *Juniperus* cf. *commums*, *J. Wallichiana*, *Rosa*—, *Ephedra*—, *Ribes*—, *Cotoneaster* species; at Tinkar down the valley to Garbyang in positions exposed to south with *Juniperus Wallichiana*, *Rosa*—, *Berberis*—, *Cotoneaster* species; near Tola-Milam 3300 m. 2 *Juniperus*—and 2 *Cotoneaster* species, whereof one with tree-like habit grows to 2 m height. *Juniperus Wallichiana* is very frequently noted; it is much used as fire-wood, "the only wood in Kuti"; it is found there up to 4600 m, and is reported not to grow from Uttadura-pass northward. There has been collected near Kuti out of the Juniperus-Wallichiana—society: *Rosa macrophylla*, *R. sericea*, *Stellera Chamaejasme*, *Hierochloa laxa*, *Scutellaria prostrata*, *Erysimum hieraciifolium*, *Myosotis* spec., *Arenaria Kashmirica*; on lime; *Callianthemum pimpinelloides*, *Anemone* spec., *Geranium* spec., *Thymus Serpyllum*; on pastures: *Iris* spec., *Polygonum affine*, *Anemone* spec., *Potentilla argyrophylla*, *Morina Coulteriana*, *Dracocephalum* spec., *Macrotomia Benthami*; near Milam-Rilkot: *Leontopodium Stracheyi*, *Anaphalis* (some species), *Cyananthus linifolius*, *Aster* spec.

The Pulsatilla—forest-steppe—belt contains in the Himalayas only few endemic species; there is for instance *Juniperus Wallichiana*, nearly related to *Juniperus pseudosabina*, moreover *Lonicera*

glauca, several species of *Nepeta* and others. The neighbouring
eastern regions have also contributed very little to this belt; here
must be mentioned the genera *Juniperus (recurva, squammata),
Adonis, Berberis, Leontopodium;* much more extensive is the
western influence which is represented by the genera *Avena, Bromus,
Pulsatilla, Erysimum, Cynanchum, Teucrium, Phlomis, Onosma,
Galium, Lactuca,* and others. This poor development of the belt
in the central Himalayas is due to the small area in horizontal and
vertical direction suited for it. In the south, the valleys are soon
too humid, and where they are dry enough, they are too hot (here
dominates *Pinus longifolia*). On the Tibetan Highland, however,
the altitudes are above what is suitable for trees. From the want
of endemics, we conclude a comparatively young age of that
belt in the Himalayas; already in the upper Indus valley, in Hindu-
kusch, in Tianschan, the belt is much more complete; but also in
the east, in eastern Tibet (up to 4500), in the mountains around
the Kukunor, in Nanschan, Alaschan, it must be much more
developed concluding from the flora-lists and descriptions of vegeta-
tion at hand. Its main-region lies between the eurasian conifer—
and steppe zones. With reference to a large part of its species,
it can be considered genetically as a xeromorphose out of the
tertiary conifer region, beginning already with the upward-move-
ment of the alpides.

The Alpine Dwarf Shrub—Tundra

(The Vaccinium uliginosum—Loiseleuria—Belt)

To conclude from the observations made by Heim and Gansser,
the birch—Rhododendron forest on the north-exposed slopes is
limited by a horizontal line, without dissolving in solitary trees.
Steep rocks and grooves of avalanches are the only interruptions
of this line. Above the forest, at about 3900 to 4000 m, begins
the region of alpine dwarf shrubs with *Rhododendron Anthopogon,
Rh. lepidotum, Rh. campanulatum, Cassiope fastigiata,* and others,
with a luxuriant growth of moss and lichens and with alpine herb
meadows. The dwarf shrub heath prefers the north-exposed
slopes, the meadows the south-exposed valley sides. Closed
Rhododendron areas are found up to about 4300 m; *Rhododendron
Anthopogon* has been seen at the Satopnath-glacier at an altitude
between 4600 and 4700 m. The ground consists of a layer of raw
humus on a very little weathered mineralic underground. The
meadows are very rich in species. On the Chaga-pass were col-
lected in a pastured meadow: *Aletris nepalensis, Allium* cf.
auriculatum, Fritillaria Stracheyi, Anemone spec., *Sedum
quadrifidum, S. crenulatum, Potentilla argyrophylla, Viola biflora*
in a very hairy form; near Kuti: *Iris* cf. *kumaonensis, Polygonum
affine, Anemone polyanthes, Potentilla argyrophylla, P. ambigua,
Geranium* spec., *Primula* div. spec., *Veronica capitata,* div. *Pedi-
cularis* spec., *Anaphalis nubigena;* on the Shialapass: *Lloydia
serotina, Orchis spathulata, Isopyrum grandiflorum, Cardamine*

pratensis var., *Potentilla argyrophylla*, *Geranium* spec., *Primula* spec., *Anaphalis nubigena*, *Aster flaccidus;* along the Ralam-pass a "paradise of flowers," with: *Juniperus Wallichiana, Meconopsis aculeata, Corydalis Gortschakovii, Potentilla* spec., *Pedicularis rhinanthoides, P. cf. macrantha, Leontopodium leontopodinum;* in the Nampa valley: *Lilium nepalense, Thermopsis barbata, Gentiana pygmaea var. acuminata, Lancea tibetica;* on a marshy bank of a brook in the west of Ralam at 4000 m: *Pedicularis tubiflora;* near Laptal, at 4200 m: *Gentiana humilis* var. *evolutior, G. Moorcroftiana, Pleurogyne carinthiaca, Pl. spathulata, Scutellaria* spec., *Aster* spec.; near Kuti, 4100 m: *Primula involucrata;* in a boggy depression on clay in the Chaga valley at 4200 m: *Veronica Anagallis, V. biloba, Parnassia pusilla;* on moraine ground of the Lebong pass: *Delphinium* spec.; on the Ralam pass: *Fritillaria oxypetala, Orchis spathulata, Corydalis* spec., *Pedicularis* spec.; in the Nampa valley: *Lloydia sorotina, Saxifraga Stolitzkae, Viola biflora, Androsace villosa var. Jaquemontiana, Lancea tibetica, Eritrichium spathulatum, Anaphalis nubigena;* on lime rubbish near Kuti: *Anemone polyanthes, Callianthemum pimpinelloides, Trigonella corniculata, Geranium* spec., *Stellera Chamaejasme, Thymus Serpyllum;* on rubbish of lime slate on the Chaga pass: *Lagotis spectabilis, Primula nivalis* var. *macrantha, Corydalis Gortschakovii;* on lime rocks near Kuti: *Juniperus Wallichiana, Isopyrum grandiflorum, Bergenia Stracheyi;* near Kalapani: *Lloydia serotina, Anemone rupicola, A. polyanthes, Saxifraga Stolitzkae, Potentilla bifurca.* On the south-exposed slopes of the inner valleys, with approach to the high plains, more and more species of the steppe are intermixed with the plant genera of the lower alpine belt (see below).

A large part of the species, and most of the genera of this lower alpine belt appear to come from the east, from east Tibet, western China and the adjoining northern East-Asia. There lies the centre of development of the Orophytes of the genus *Tofieldia, Rheum, Meconopsis, Delphinium, Rhododendron, Gentiana, Primula, Androsace, Swertia, Pedicularis, Aster, Leontopodium, Saussurea,* and others, while the relations to the north-west are much less numerous. Plant societies of the belt dominated by Ericaceae are completely missing in the central- and west-Asiatic mountains (in the Tianshan and Altai they are seen only on the northern mountain side), and are found again only in the caucasus, in the Balkan and in the Alps. The belt of *Vaccinium uliginosum, Loiseleuria* is found in the central Himalayas as a small zone, enlarging fan-like towards the east, over the east-Asiatic mountains to the arctic plains, where it encloses the northern hemisphere in a large zone poor in species, limited in the south by the subarctic forest. Towards the west, the belt enlarges also, but is found only island-like and very poor in the central- and west-Asiatic mountains, becoming again more complete in the caucasus and in the European mountains.

In the Himalayas the belt is autochthonous; that must be concluded from the genetic relations to species of lower regions (Diels 1901), and from the numerous Endemics of high value. Just as in the other areas of its original regions, it is a production of orophyts from the conifer-zone with older parts from the tertiary angiosperm forest regions, beginning with the upward-movement of the alpides. Richness in species and the genetic union of the flora-types with the flora of the lower regions show distinctly that the Eurasiatic mountains, mainly the east-Tibetan-Chinese mountains, and not the arctic circle, are the centre of origin of this flora as has already been mentioned by *Grisebach* 1872.

The influence of man in the lower Alpine region is unimportant, and is only found by pasturage near the caravan paths.

The Carex—Elyna—Belt

The meadows of the highest vegetation belt expand above the dwarf-shrub heath and the luxuriant meadows of the lower Alpine zone, at an altitude of about 4300m. This belt is found in pioneer turf up to 4900m and in single individuals upto 5500m. The snow limit is found by Heim and Gansser to be in the inner valleys at 5300 to 5500m, near the Tibetan frontier at about 5600m. In this upper Alpine region, there is not only the upper thermic limit for phanerogamic plants, but also the dry-limit, that is the limit beyond which all precipitation is evaporated again, without deposition downwards. On the Himalayan side of the mountain passes, there are still flourishing continuous turf societies, interrupted only by rubbish or rock; on the Tibetan side, they are limited to flat moulds containing ground-water and to the banks of periodical watercourses. Here, the vegetation is dominated by Gramineae and Cyperaceae. In such places was still found brown-black humus, as for instance at the *Tinkar-Lipu*—pass, 4760m, and in a depression near by, at 5100-5200m, boggy soil with *Braya rosea, Draba alpina, Ermania himalayensis, Lagotis* spec., and plenty of *Ranunculus pygmaeus*, (found here for the first time in the Himalayas), as well as *R. nivalis*, which, up to the present, was considered as chiefly arctic. Below the pass, between 4500 m, there were observed in the turf: *Oxygraphis glacialis, Ranunculus pygmaeus, Sedum* of the Tribus *himalensis, Saxifraga Stoliczkae, Potentilla* spec., *Primula nivalis* var. *macrantha;* at Amlang-La, 4700 m, in a meadow above groundwater: *Sedum* spec., *Oxytropis* spec., *Primula tibetica, Pedicularis Oederi, P. rhinanthoides, Microula Benthami, Taraxacum dealbatum, T. leucanthum, Aster* spec.; near Junglea, spongy meadow with: *Oxytropis proboscidea, O. microphylla, Gentiana humilis* var. *evolutior, Saussurea* spec., *Taraxacum leucanthum;* on the Ralam pass, at 4600 m: *Juncus leucomelas, Anemone* spec., *Saxifraga* cf. *nana, S. sibirica, S. pallida, Gentiana Wardii, G. tubiiflora, Leontopodium* spec., *Anaphalis nubigena;* near the Satopnath glacier in turf at 4600 m: *Gentiana nana* and *G.* cf. *depressa* ("all the rest dry," 7 X. 1936);

Dolma-La at 5100 m on a level meadow; *Parrya exscapa, Veronica*
pec., From the Uttadura-pass Heim writes that there was only
" hard green, stuffed plants, mosses, dwarf enzian"; 400 goats and
sheep out of 800 were killed by bad weather lasting five days and by
want of food. On moraine and rubbish were collected at the
Lipu Lek—pass, between 4700 and 5200m: *Polytrichum—, Dicra-*
*num—, Hedwigia—, Tortella—*species, *Ranunculus pygmæus,*
Draba spec., *Sedum* of the Tribus *himalensia, Chrysosplenium*
carnosum, Saxifraga setosa, Primula minutissima var. *spathulata;*
near Kuti on rock and rocky rubbish at 4800m: *Saxifraga sibirica,*
Gentiana capitata, Androsace villosa var. *villosissima, Primula*
nivalis var. *macrophylla;* near the Raksaslake, at 4600m: *Arenaria*
musciformis, Dracocephalum heterophyllum, Marrubium spec.,
Allardia tomentosa; at Dolma-La, south side, 5500m, between
granite blocks: *Urtica* cf. *hyperborea, Draba altaica, Potentilla*
spec., *Astragalus Heydei, Astr. confertus, Veronica* cf. *bilboa,*
Leontopodium nanum; at Mangshang-La, between 4700 and 4850m,
on dry rubbish: *Sedum fastigiatum, Oxytropis* spec., *Eritrichium*
strictum, Pedicularis globifera, Aster flaccidus, Leontopodium
leontopodinum, Senecio arnicoides, Allardia tomentosa, Saussurea
scrocephala; at Amlang-La, 4700m on rubbish: *Hedinia tibetica,*
Torularia humilis, Pedicularis spec., at the Ralam pass on rubbish:
Rheum spec., *Saxifraga flagellaris, Allardia glabra, Anaphalis*
nubigena; there also at 4600 m: *Wahlenbergia gracilis;* at the Kian-
gur pass, between 4600 and 4900m: *Delphinium* spec., *Saxifraga*
flagellaris, Gentiana aquatica, Pleurogyne brachyanthera, Microula
Benthami, Lamium rhomboideum; in the *Chidamu-gorge* at 5000m:
Gentiana frigida. var. *nubigena, Leontopodium monocephalum;* in
the eastern Lap valley on flysch back between 5000 and 5200 m:
Gentiana frigida var. *nubigena, Senecio* cf. *retusus;* near Bal-
chadura between 5200 and 5300m: *Gentiana nana, G. frigida* var.
nubigena, Nepeta longibracteata, Pedicularis globifera; at the
Balchadura—pass at 5400m on rubbish: *Potentilla fruticosa* var.
pumila; and on moraine at 4900-5000m: *Thylacospermum rupi-*
fragum in cushions of up to 2·5m diameter; at 5450m still *Senecio*
arnicoides; along the Chirdum pass, 5300m: *Saxifraga viscidula;*
at the Chalda pass, 5300m, *Delphinium vestitum;* on the Lipulek
pass on rocks between 4800 and 5300m: *Stereocaulon—,Evernia—,*
Cladonia species, *Draba setosa, Saxifraga imbricata, S. Stolitzkae,*
Chrysosplenium carnosum; on a rocky ridge near Kuti, 4800m,
Corydalis cachemiriana.

The Carex—Elyna belt possesses in the innermost Himalayan
chain rich *fittings* of species, genetically bound to the flora of
lower regions; nevertheless it contains a very strong falling gradi-
ent of flora form the Tibetan-Chinese mountains between Yünnan
and Kukunor; in the frontier region near Tibet, their number falls
down to a few hundred species. Genera as *Eutrema, Ptilotrichum,*
Parrya and others indicate central Asiatic influence. Old highly
valued endemics are frequent. Their number increases by itself
with the altitude, owing to increasing isolation and specialisation.

They can follow much less all displacements, due to change of climate, than species in the lower belts. These Orophytes are remarkable especially on account of their xeric development. High mountains and desert-climate at the same time produce forms like that of *Saussurea gossypiphora, S. tridactyla*, with stem and foliage covered with a mantle of hair like wad. Many of these characteristic species are widely and equally spread in Tibet and the adjoining Himalayan valleys, *e. g. Arenaria musciformis, Thylacospermum rupifragum, Microula Benthami, Lancea tibetica, Nepeta longibracteata, Aster flaccidus*, and others.

The centre of development of the Carex—Elyna belt lies in the central-Asiatic mountains; the north-American, as well as the European mountains are much poorer. From Central-Asia it has reached, chiefly over the east-Asiatic mountains, the arctic circle, and from there it has sent again during the glacial periods many species to more southern mountains.

The Stipa-Steppe—Belt

At the altitude of the subalpine forest already, in fragments of forest-steppe, on south-exposed dry rocks, and then high up in the upper Alpine region, Heim and Gansser have collected and noted some solitary Stipa-steppe types. Very frequent is *Ephedra Gerardiana* var. *Wallichii; Potentilla fruticosa* climbs as *P. biflora* near Kuti up to 5000 m; *Trigonella corniculata, Carex supina* seem to be rather frequent. Near the Kiangur, they found *Lamium rhomboideum*, near Kuti *Arenaria kashmirica* and *Morina Coulteriana.* Unfortunately, Gramineae and Cyperaceae, which are so important for the recognition of grass societies, have not been collected. All species of this kind, especially the representatives of half-deserts and deserts, not seldom on the Tibetan plain, come from lower regions but they can withstand, owing to their xeric structure, the extreme climate of the higher belts. The Stipa-steppe is found in eastern Tibet and in China, in the north of Tsinling-Shan, to quite a remarkable extent, but the major part of the species in Kumaon and the adjoining Tibet come from north and west (e.g. species of *Elymus, Carex, Stipa, Caragana, Scutellaria, Nepeta, Artemisia,* and others). The number of species, and the number of endemics of these belts is relatively small. It increases towards west and north-west, just the reverse of the Carex —Elyna and the *Vaccinium uliginosum*—Loiseleuria belts. The genetic relations also point to the west; these three xeric belts must be considered therefore as a recent contribution to the central Himalayas. There reaching the upper Alpine regions, and the absence of all forest belts, even of the lower Alpine belts, is a phenomenon repeated only in the Bolivian Andes (with ecological equivalents).

Collections of plants have been made only in the Alpine regions, over 4000 m, but ample notes of Heim give some indications about the vegetation in lower areas. There seems to be no spruce belt, as in middle and eastern Europe and in parts of northern Asia, between subalpine, subarctic and angiosperm forests; this acidiphile vegetation, demanding a moderately cool climate with ample precipitation, does not find these conditions in the central Himalayas. The beech belt is represented by forests, rich in herbs of *Abies Webbiana*, resp., *A. Pindrow, Cupressus torulosa, Taxus baccata, Ilex* spec., *Quercus cf. semicarpifolia, Hedera Helix*; in the undergrowth near Shankula: *Polygonatum verticillatum, Circaea lutetiana, Fragaria vesca, Oxalis Acetosella, Rubus, Impatiens, Ferns, Rhododendron*. The best development is attained on north-exposed slopes, between 2500 and 3000 m. Below we find, just as in Europe, and eastern Asia, a region very rich in woody plants, the belt of Quercus—Tilia—Acer, with deciduous leafy oaks, *Aesculus indica, Juglans regia*, 5 *Acer* species *Picea Morinda*, in damp places between 2000 and 2500 m, and below, the evergreen subtropical angiosperm forest, interrupted on long distances by the more drought-resisting *Pinus longifolia* vegetation.

There remains to be done still much hard and patient work in floristics, systematics and phytocenology (especially concerning the small geographic races), before we can succeed in writing a description of this beautiful world of plants in those stupendous mountains.

Literature

DIELS, L. Die Flora von Zentral-China, Leipzig 1901.

HOOKER, J. D. The flora of British India, London, Vols. 1-7, 1875-1897.

HANDEL-MAZZETTI, H. von, Symbolae Sinicae, Wien 1929.

HEMSLEY, W. B. and PEARSON, H. W. The flora of Tibet or High Asia in "Journ. Linn. Soc.," Bot., Vol. XXXV, 1902.

LIMPRICHT, W. Bot. Reisen in d. Hochgeb. Chinas u. Osttibets in "Fedde, Rep.," Bd. XII, 1922.

PAMPANINI, R. La flora del Caracorum in "Spedizione italioma De Fillippi" Ser. II, Vol. 10, Bologna, 1930.

SCHMID, E. in BOSSHARD, W. Bot. Ergebnisse d. Deutschen Zentr. As. Exped. 1927-28, Fedde Rep., Bd. XXXI, 1932.
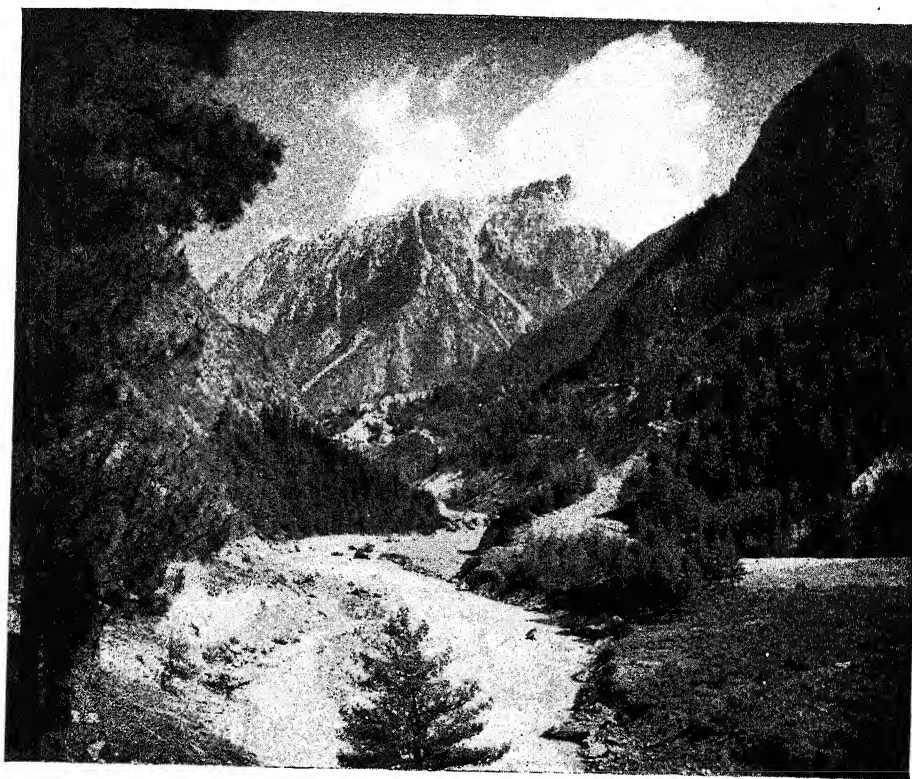
Photo A. Heim

Kali River above Garbyang; view towards N. E. 3,050 m. Probably *Pinus excelsa.*

Photo A. Heim

The sacred birch near Tijang, Lissar valley 3,300 m.

Photo A. Heim

Upper forest—limit near Kuti, 3,800 m. with *Betula utilis.*

ACTINOMYCES: THEIR BIOCHEMICAL RE-ACTIONS AS AIDS IN THEIR CLASSIFICATION PART I. REDUCTION OF NITRATES

BY

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In this paper the generic name *Actinomyces* has been used to include not only the ray-fungi but also those grouped under *Discomycetes, Streptothrix, Nocardia,* etc., in the sense as accepted by Breed and Conn (1), Ford (2), and Topley and Wilson (3). Objections are likely to be raised because many prominent authorities do not agree to this arrangement. The task of placing the vast number of these fungi into their proper generic position and then of identifying each of them has been taxing the mycologist for quite a long time. Many well-known workers in different countries have made valuable contributions towards the classification, but scientists are still in disagreement as to their proper classification. It is no wonder that none of the methods and schemes suggested so far have answered the requirements, since it is not easy to put so many organisms (numbering about six thousand) into their proper genera and species.

Dreschler (4) was probably one of the first to attempt a systematic classification from the morphology of these fungi but his methods were complicated and very difficult to follow. The slide-impression and camera-lucida study of the fungi does not seem to be a satisfactory method, and the grouping of so many fungi is not possible even with such a highly technical method.

On the whole, the most practicable scheme yet published is that of Orskov(5), and his method has been followed by Miss Dagny Erikson (6) in classifying the national collection of type cultures maintained by the Medical Research Council at the Lister Institute in London. But a perusal of her work does not help us much as regards an easy method of putting these fungi first into different groups and sub-groups before passing to the individual species. Great difficulties seem to arise when a medical man isolates a fungus from a patient and wants to find out whether it is a new species, and whether it is pathogenic or not.

Waksman in America has made a detailed study of *Soil Actinomyces* and has tried various biochemical reactions for them, such as *tyrosinase reaction, formation of pigments in media containing proteins, reduction of nitrates to nitrites* with different sources of carbon, etc.

The present investigation was taken up some time ago at the suggestion of the late Col. H. W. Acton with the idea that this biochemical reaction might prove to be a useful factor in the grouping of a large number of these fungi. The type cultures, maintained in the School of Tropical Medicine, Medical Mycology Enquiry under Dr. P. A. Maplestone, have been studied in these experiments and we are grateful to Dr. Maplestone for kindly giving us all the facilities for the work.

Experimental

The media used for the culture of the Actinomyces was the synthetic liquid media suggested by Czapeck which was composed as follows :—

Saccharose 30 grams, dipotassium phosphate 1 gram, potassium chloride $0 \cdot 5$ gram, magnesium sulphate $0 \cdot 5$ gram, ferrous sulphate $0 \cdot 01$ gram, sodium nitrate 2 grams, and distilled water to make 1,000 c.c. It was adjusted to Ph $6 \cdot 5$ sterilised.

In the preliminary experiments, the media inoculated with the fungus were tested qualitatively from day to day for nitrite with the help of sulphanilic acid and—naphthylamine acetate. It was found, however, that even the controls gave a strong reaction for nitrites. It was at first thought that probably the ferrous sulphate present was causing a reduction of the nitrate. The ferrous sulphate was therefore omitted from the media and the experiments repeated.

This did not, however, remove the difficulty and so it was decided to estimate the nitrite carefully by a quantitative method. The ferrous sulphate was, however, omitted in all the later experiments.

About 15 c.c. of the liquid media was put into each of a series of 12 test tubes. Six of these were inoculated and the other six kept for control. As far as practicable, the minimum amount of fungus, that could be taken at the point of the inoculating chromium wire, was used for the inoculation. The tubes were kept at about 37° C. and one tube of each was tested every week, along with the controls. In case the controls showed strong reduction, the whole series was rejected. Two series of experiments have been carried out with each species, and those fungi which gave uniform results in both the series have been considered here. The results of some, which could not be confirmed by the second experiment owing either to the growth being too poor or the culture being contaminated, have been rejected.

The method of estimating the nitrite formed was the standard one recommended for its estimation in water analysis (8) and is described briefly as follows :—

Reagents—(*a*) 8 grams of purest sulphanilic acid dissolved in one litre of 5 N acetic acid; (*b*) 5 grams of—naphthylamine dissolved in one litre of 5 N acetic acid. (*c*) *Sodium nitrite stock solution,* prepared by dissolving $1 \cdot 1$ grams of silver nitrite in nitrite-free water, precipitating the silver with sodium chloride solution and diluting to one litre, (*d*) *Standard sodium nitrite solution,* prepared by dissolving 100 c.c. of the stock solution (*c*) to one litre and then diluting 30 c.c. of this solution to one litre with nitrite-free water, adding one c.c. of chloroform and preserving in small well-stoppered amber-coloured bottles. 1 c.c. of this solution is equivalent to $0 \cdot 001642$ mg. of NO_2.

TABLE I

No.	Name of the fungus.	Reduction of Nitrate (1st Series).			Reduction of Nitrate (2nd Series).			Remarks.
		1st week.	2nd week.	3rd week.	1st week.	2nd week.	3rd week.	
1	Actinomyces carnosus (Millard).	1·5	0·8	1·5	0·8	0·2	0·45	No.
2	,, cellulosae (Krainsky)	0·5	0·5	0·3	0·35	0·18	0·15	,,
3	,, citrius (Gasperini)	0·3	—	—	0·35	0·2	0·25	,,
4	,, ceoelicolor (Miiller)	0·15	0·1	0·5	0·17	0·25	0·25	,,

TABLE I—(*continued*)

No.	NAME OF THE FUNGUS.	REDUCTION OF NITRATE (1st SERIES).			REDUCTION OF NITRATE (2nd SERIES).			REMARKS.
		1st week.	2nd week.	3rd week.	1st week.	2nd week.	3rd week.	
5	Actinomyces diastaticus (Krainsky)	0·1	0·1	0·1	0·18	0·2	0·2	No.
6	,, flavovirens (Waksman)	0·1	0·1	0·1	0·18	0·2	0·23	,,
7	,, bovis (Harz)	0·1	0·1	0·15	0·5	0·2	0·27	,,
8	,, Halstedi (Waksman and Curtis)	0·3	0·1	0·2	0·15	0·1	0·35	,,
9	,, fradii (Waksman and Curtis)	0·2	0·2	0·1	0·18	0·22	0·25	,,
10	,, maculatus (Millard)	0 4	0·1	—	0·2	0·35	0·4	,.
11	,, reticuli (Waksman and Curtis)	0·2	0·4	0·1	0·45	0·3	0·5	,,
12	,, verne (Waksman and Curtis)	0·4	0·1	0·3	0·3	0·2	0·4	,.
13	,, xanthostroma (Wollenweber)	0·2	0·2	0·2	0·2	0·1	0·15	,,
14	,, chromogenus (Gasp)	0·6	0·1	0·15	0·3	0·12	0·15	,,
15	,. alboflavus (Waksman and Curtis)	0·1	0·15	0·5	2·5	0·75	0·25	,,
16	,, cabies (Gussow)	1·75	0·44	0·4	0·3	0·5	0·4	No.
17	,. viridochromogenus (Krainsky)	1·25	2·0	—	4·5	1·0	1 1	Moderate.
18	,, griseolus (Waksman)	0·1	0·25	0·4	0·26	0·85	0·54	,,
19	,, clavifer (Millard)	2·2	4·5	3 5	0·55	0·9	0·38	,,
20	,, keratolytica (Acton & Mcguire)	1·0	0·6	9·0	6·0	4·6	1·35	,,

TABLE I—(*continued*)

No.	Name of the fungus.	Reduction of Nitrate (1st Series).			Reduction of Nitrate (2nd Series).			Remarks.
		1st week.	2nd week.	3rd week.	1st week.	2nd week.	3rd week.	
21	Actinomyces lavendulae (Waksman and Curtis)	1·9	1·5	8·0	3·2	1·3	5·0	No.
22	,, Bobili (Waksman and Curtis)	1 25	2 25	3·25	4·5	5·0	5·0	,,
23	,, aureus (Waksman and Curtis)	1·5	1·5	4·5	3·0	3·25	3·0	,,
24	,, albus (R. D.) (Gasp Var Ochroleuis)	4·0	4·5	3·5	1·75	2·0	2·0	,,
25	,, albus (Krainsky var. a. Cif)	4·5	4·5	3·5	2·5	9·0	7·0	,,
26	,, Madurae (Lackner)	2 25	18	10	7·75	8·0	7·0	,,
27	,, microflavus (Krainsky)	1·4	2·25	8·0	12·0	11·5	13·0	,,
28	,, nigrificans (Krug) Wr.	600	600	750	275	375	450	
29	,, tricolor (Wollenweber)	650	725	850	250	75	30	
30	,, tyrosinaticus (Krainsky)	1,500	2,400	2,700	150	55	150	
31	,, violaceus ruber (Waksman and Curtis)	750	800	750	300	225	100	

Method—A measured volume of the sample was placed in a standard 50 c.c. Nessler tube and diluted to 50 c.c. A set of standards were prepared in 50 c.c. Nessler tubes by diluting various amounts of standard nitrite solution to 50 c.c. with nitrite-free water. 1 c.c. of sulphanilic and solution and 1 c.c. of—naphthylamine acetate solution were added to the sample and to each standard. They were mixed thoroughly and allowed to stand for 10 minutes. The samples were not allowed to stand for more than 30 mins. before making the comparison.

The figures shown in the following table indicate the volume in c.c. of the standard nitrite solution equivalent to 5 c.c. of the samples tested. Judging from the controls, the figures below 0·5 c.c. were regarded as *negative,* figures between 0·6 and 20 c.c. as *moderate* and those above 20 as *strong.*

TABLE II

(Controls)

No.	NUMBER OF CONTROLS.	REDUCTION OF NITRATE (1st SERIES).			REDUCTION OF NITRATE (2nd SERIES).			REMARKS.
		1st week.	2nd week.	3rd week.	1st week.	2nd week.	3rd week.	
1	C_1	0·05	0·05	0·1	0·2	0·8	0·6	No.
2	C	0·1	0·05	0·1	0·1	0·6	0·5	,,
3	C_3	0·2	0·1		0·1	0·1	0·2	,,
4	C_4	0·1	0·1	0·15	0·2	0·5	0·48	,,
5	C_5	0·1	0·1	0·1	0·15	0·17	0·35	,,
6	C_6	0·1	0·1	0·1	0·1	0·15	0·18	,,
7	C_7	0.2	0·15	0·25	0·1	0·18	0·4	,,
8	C_8	0·05	0·15	0·1	0·1	0·3	0·5	,,
9	C_9	0·1	0·5	0·2	—	—	—	,,
10	C_{10}	0·15	0·25	0·25	0·1	0·3	0·35	,,
11	C_{11}	0·25	0·1	0·7	0·27	0·3	0·2	,,
12	C_{12}	0·3	0·1	0·1	0·1	0·1	0·1	,,

Discussion of Results—It was found that the maximum reduction was usually obtained at the end of the third week. With some, it was the second week, and with others, the 4th or 5th week, but the end of the third week was found to suit most. In some species, after the maximum reduction in the third week the reduction

gradually became less and less. The results may be divided into three main groups: (*a*) those showing strong reduction, (*b*) those showing moderate reduction and (*c*) those showing very little or no reduction. The results allow us to take this factor as an aid in dividing this large·group of fungi into sub-groups.

Suggestion of a Scheme—Brumpt (9) has suggested the following scheme which is based·upon the ideas and nomenclature of Vuillemin (10), Pinoy (11), Fullerton (12) and Langeron (13) :—

Microsiphonales—Hypomycetes with fine mycelial filaments not segmented, diameter-one micron or less without distinct nucleii.

Cohnistretothrix—Difficult to cultivate, generally anaerobic but at times an aerobic, not producing spores in cultures.

Actinomyces—Generally aerobic but some can grow anaerobically, forming spores.

We venture to put forward the following scheme:—

Actinomyces

1. Producing coloured pigment in all media containing proteins (chromogenous).

2. Not producing pigment in protein media (Nonchromogenous).

3. Young mycelia, acid fast.

4. Young mycelia, not acid fast.

5. Proteolytic on inspissated serum (gelatine is not suitable for tropical countries).

6. Not proteolytic.

7. Haemolysis in blood-agar medium.

8. No haemolysis.

9. Reducing nitrates to nitrates (moderate and strong).

10. No reduction.

11. Milk-clotting or hydrolysis.

12. No change.

13. Branching—Racemose type.

14. Branching—Dichotomous.

15. Spores or conidia abundant.

16. Growth by disintegration of the mycelial filaments into oidia.

Summary

The classification of the group of micro-organisms, generally designated as Actinomyces, is still very unsatisfactory and various authors have put forward different schemes of classification based on the morphology, clinical characters physiological properties, etc. The authors have studied the biochemical property of the reduction of nitrates to nitrites and have been able to divide them into three groups. They think that further work on these lines would provide additional factors in their classification.

References

1. Breed, R. S. and Conn, H. J. (1919).—Jour. Bact. 4, 585-602.
2. Ford, W. W. (1927).—Text book of Bacteriology.
3. Topley and Wilson (1929).—Principles of Bacteriology and Immunity.
4. Dreschler, C. (1919).—Bot. Gaz. 67, 65-83, 147-168.
5. Orskov, J. (1923).—Investigations as to the morphology of the Ray-fungi. Copenhagen.
6. Dagny, Erikson (1935).—Medical Research Council Special Report, series No. 203.
7. Waksman, S. (1919).—Soil science 8, 71-215.
8. Amer. Pub. Health Assc. (1933).—Standard Methods for the examination of Water and Sewage. 7th Edition 1933.
9. Brumpt (1936).—Precis de Parasitologie.
10. Vuillemin, P. (1910).—Compt. Rend. Acad. des Sci. Vol. 150, p. 882.
11. Pinoy, E. (1913).—Bull. Inst. Pasteur, 11, 928, 977.
12. Fullerton, A. G. B. (1910).—Lancet I, 555, 626, 769.
    ————— (1912).—Brit. Med. Jour. I, 300.
13. Langeron (1925).—Nouveau Traite de Medicine 4, 445.
14. Castellani, A.J. (1929).—Jour. Trop. Med. (Hyg.), 32, 1-8.

A SHORT NOTE ON THE
RATE OF RESPIRATION AND RESPIRATORY
QUOTIENT OF STARVED LEAVES OF
ARALIA SP. BEFORE AND AFTER A COURSE
IN NITROGEN *

BY

A. B. SARAN, M.Sc.

Introduction

It has been demonstrated by several workers that respiration in an atmosphere devoid of oxygen is quite distinct from that in air. Blackman and Parija (1, 2, 5) working on apples have shown that when nitrogen is substituted for air, the respiratory activity rises for a few hours then falls down gradually to the previous air line value. In certain apples, on the other hand, the effect of nitrogen has been to decrease the rate of respiration. But in all cases, when air was admitted after a period in nitrogen, the rate of respiration was enhanced, which however does not surpass the one in air. This sort of behaviour exhibited by a respiring organ, when subjected to a period in nitrogen is not quite common. Inamdar and Singh (3) working on leaves from tropical plants did not come across such phenomenon. It was, therefore, desired to investigate the behaviour of leaves of an *Aralia sp.* subjected for a known period in pure nitrogen and their respiratory ratio (CO_2/O_2) before and after the nitrogen treatment.

Material and Method

The leaf: All the leaves required for this investigation were taken from a single plant growing practically in shade. Soon after isolation, invariably done in the morning, the leaves were quickly weighed and kept in the respiration-chamber, with their petioles dipping in water.

Estimation of CO_2: The rate of respiration was estimated by the continuous current method at a controlled temperature of $28 \cdot 5°C$. Two-hourly output of CO_2 was measured by tritating the baryta

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* This work was undertaken and completed at the Botanical Laboratory, Ravenshaw College, Cuttack.

GRAPHS 1-3. SHOWING THE AIR AND NITROGEN RESPIRATION OF
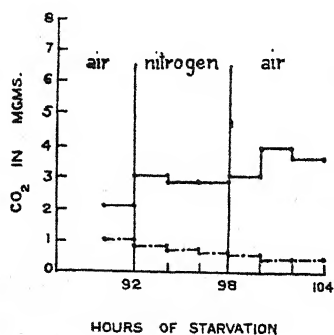THE SAME LEAF AT VARIOUS HOURS OF STARVATION.

GRAPH 1.
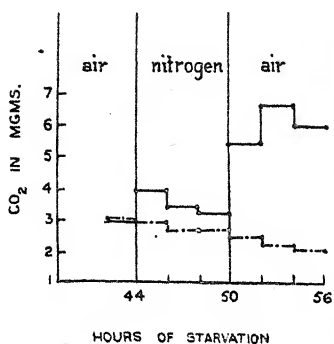
GRAPH 2.
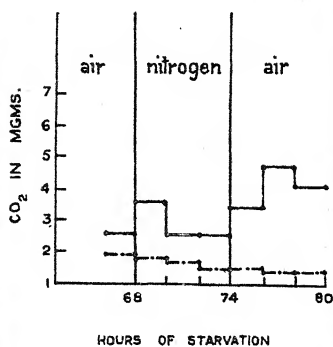
GRAPH 4. SHOWING THE AIR AND
NITROGEN RESPIRATION OF LEAF
STARVED FOR 22 HOURS.

GRAPH 3.
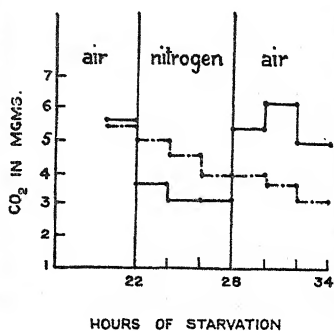
——·—— Experimantal leaf.   —·—·— Control leaf : respiration
in air throughout

from the pettenkofer tubes against standard hydrochloric acid solution and the result was expressed for 10 gms. of fresh weight of leaves.

Source of nitrogen: Commercially prepared nitrogen obtained under pressure in cylinder was used throughout. Before using, it was tested for oxygen and was found to be free from it.

Estimation of the respiratory quotient CO_2/O_2: The amount of oxygen taken in by the leaves and carbon dioxide given out during the period was determined to calculate the respiratory quotient by analysing the respiration gas in Haldane's (4) gas analysis apparatus. Estimation of CO_2 by this method served as well a check for the amount of CO_2 obtained from the titration of the baryta from the pettenkofer tubes.

Results and their Consideration

Initial output of CO_2: The initial output of CO_2 was not the same for all the leaves experimented upon. In some it started at a high pitch, whereas in others it was rather low. The highest initial output of CO_2 was 13·6 mgs. per two hours and the lowest being 6·4 mgs. per two hours, per 10 gms. of fresh leaf. Although the experiments were carried out in different seasons no marked seasonal variations on the initial rate of CO_2 output was noticed except in the month of March, when higher values were invariably met with.

Normal course of respiration: The respiratory activity of the isolated leaves ˙ start at a high level, which during the subsequent hours of starvation takes to a descending course. It is not uncommon, on the other hand, to find, specially when the leaves have been starved beyond 40 hours or so, a rate of respiration more or less constant for a period of about 3-4 hrs.

Respiration in nitrogen:

(*a*) *Subjecting leaves to nitrogen at 44 hours of starvation*: Respiration of 44-hours-starved-leaves studied in nitrogen for 6 hours, yielded interesting results. The first two hourly readings, showed a higher value (vide Fig. I, graph 1) than the one in air immediately before nitrogen. The second and the third two-hourly readings, however, presented values which were lower than the first one, but distinctly higher than that of the one previously in air. After 6 hours course in nitrogen, when air was again admitted, the first two-hourly value for respiration surpassed much beyond the one in air. This again was followed by a rate still higher in value as is evident from the second two-hourly reading. The subsequent readings, however, showed falling values. The same leaf when subjected again to nitrogen at 68 and 92 hours of starvation for a period of 6 hours each, yielded similar results (Fig. I, graphs 2 & 3) except that the "after-effect" of nitrogen (*i.e.* the difference between the original and the subsequent highest air values after a course in

nitrogen) gets smaller (Table I, Cl. H) as the period of starvation advanced.

(*b*) *Subjecting leaves to nitrogen at 22 hours of starvation*: At this hour of starvation, respiration of leaves in nitrogen yields, unlike the previous case, falling values (Fig. I, graph 4) as compared to the one immediately before in air. Enhanced rate of respiration, however, was noticed when air was again let in, *i.e.* the first two-hourly readings rose as far as to the previous air line value, whereas the second one actually surpassed it. The subsequent readings, however, presented falling values. The "after-effect" in this case, curiously enough is much smaller than those obtained from leaves starved for 44, 68 or 92 hours.

The respiratory quotient CO_2/O_2: The respiratory quotient of the starving leaves, before and after nitrogen treatment, was determined in each case. Before subjecting the leaves to nitrogen the quotient was always found to be unity. For determining the quotient after a course in nitrogen the respiration-apparatus had simply to be run for two hours so as to free it completely of any traces of nitrogen. It was, therefore, from the next two-hourly readings that the quotient was actually determined, which curiously enough came only up to $\cdot70$-$\cdot75$, *i.e.* the leaves had taken in more of oxygen than the amount of CO_2 given out. This abnormal value, however, during the next two hours, returned to normal, *i.e.* unity.

What is the ultimate fate of the extra amount of oxygen taken in by the leaves, which results in lowering the respiratory ratio is a problem difficult to explain. It is highly probable that during the period the leaves are put to respire in nitrogen, all traces of oxygen within the leaf tissues is made available and thus used up in the respiratory processes. When air is again admitted this loss of oxygen from the leaf tissue is probably made good, through a slow process leading thereby to the utilization of a little more quantity of oxygen than that was actually needed for the quantity of CO_2 produced, which thus seems to be responsible for temporarily lowering the respiratory quotient.

The peculiar respiratory behaviour of the leaves in nitrogen as well as in air soon after, does not require any further ellucidation than what has already been advanced by Blackman and his co-workers (1, 2, 5) for a similar phenomenon in apples upon which they experimented.

Summary

No marked seasonal variation has been observed to exist in the initial rate of respiration of the leaves of *Aralia* sp:

When nitrogen is substituted for air at or beyond 44 hours of starvation the rate of respiration gets temporarily enhanced, but the same treatment given at an earlier stage (*i.e.* at 22 hours of

starvation) leads to the lowering of the respiration rate. In both the cases, however, when air is again admitted after a course in nitrogen, the rate of respiration is found to shoot up temporarily.

After a course in nitrogen, the respiratory ratio presents a low value of ·70 to ·75 for about a period of 4 hours beyond which it returns to its normal value, *i.e.* unity.

Acknowledgment

This problem was very kindly suggested by Professor P. Parija, M.A., I.E.S., to whom the author is indebted for his criticisms, help and suggestions during the course of this investigation.

Literatures

1. BLACKMAN, F. F. (1928).—Proc. Roy. Soc. (Lond) 103, B.

2. BLACKMAN, F. F. and PARIJA, P. (1928).—Proc. Roy. Soc. (Lond.) 103, B.

3. INAMDAR, R. S. & SINGH, B. N. (1928).—Jour. Ind. Bot. Soc. Vol. VI.

4. HALDANE, J. S. (1920).—Method of Air Analysis.

5. PARIJA, P. (1928).—Proc. Roy. Soc. (Lond.) 103, B.

TABLE I

Showing the respiration of the leaves of *Aralia* sp. in air as well as in nitrogen at various hours of starvation.

Experimental leaves.	Respiration in air immediately before nitrogen.	Respiration in nitrogen.			Respiration in air immediately after 6 hours course in nitrogen.			The 'After-effect.' F − A	REMARKS.
		First two-hours.	Second two-hours.	Third two-hours.	First two-hours.	Second two-hours.	Third two-hours.		
	A	B	C	D	E	F	G	H	
Starved for 44 hours ..	2·82	3·90	3·47	3·25	5·42	6·64	6·00	3·82	The same leaf was subjected to nitrogen for six hours each at 44, 68 and 92 hours of starvation.
Starved for 68 hours ..	2·60	3·68	2·60	2·60	3·47	4·77	4·10	2·10	
Starved for 92 hours ..	2·17	3·03	2·82	2·82	3·01	3·98	3·61	1·81	
Starved for 22 hours ..	5·68	3·61	3·27	3·27	5·42	6·37	4·99	0·69	The leaf was subjected to nitrogen for 6 hours at 22 hours of starvation only.

Control leaves.	Respiration of control leaves in air at the various periods of starvation corresponding to the experimental leaves.							Remarks.
	A	B	C	D	E	F	G	
Starved for 44 hours ..	2·84	2·84	2·61	2·61	2·40	2·23	2·00	Respiration of the same leaf was studied from 44 hours to 104 hours of starvation.
Starved for 68 hours ..	1·96	1·86	1·63	1·40	1·40	1·31	1·31	
Starved for 92 hours ..	1·00	0·80	0·80	0·63	0·52	0·43	0·43	
Starved for 22 hours ..	5·48	5·00	4·60	3·92	3·92	3·60	3·27	Respiration of the leaf was studied from 22 hours to 44 hours of starvation.
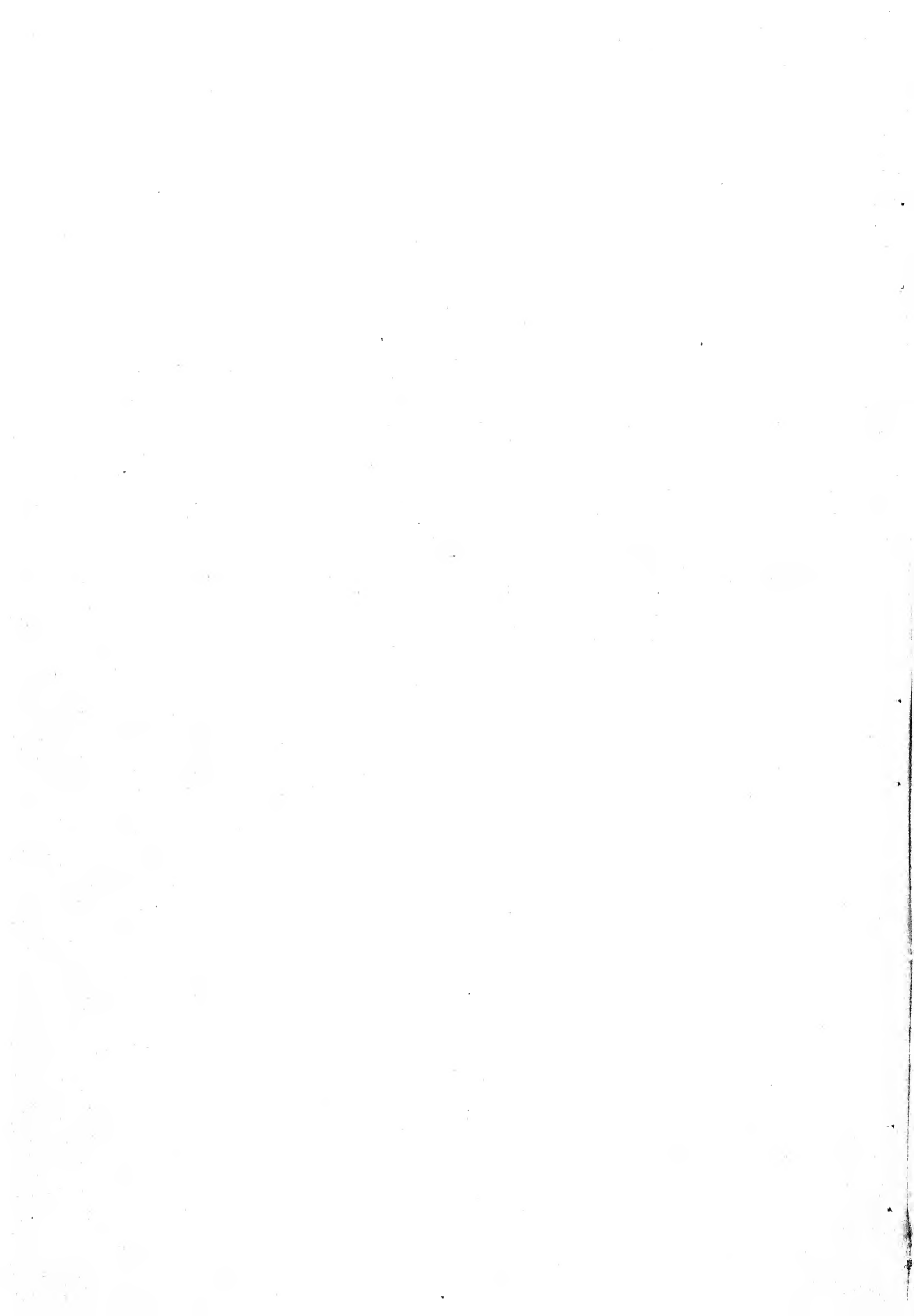
ON THE MORPHOLOGY, CYTOLOGY AND PARASITISM OF *UROMYCES HOBSONI* VIZE. (*U. CUNNINGHAMIANUS* BARC.)

(A PRELIMINARY NOTE)

BY

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Communicated by M. A. Sampathkumaran

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Introduction

As far back as 1891, Barclay reported on the life history of this rust fungus in Saire (Simla), and noted its peculiar development. Ajrekar and Parendakar (1931) carried out a detailed investigation of this fungus on *Jasminum grandiflorum*, and its relation to a *Uromyces* species on *J. malabaricum*. In a detailed study of the spore forms by the writer, various interesting features in the life cycle were met with, which have not been recorded in rusts in general.

Sori-forms and their development

The autoecious nature of the rust is evident from the fact that all the three sori-forms, *viz.*, pycnidia, aecidia and telia are found on the same plant, *Jasminum grandiflorum*. Uredospores are absent.

1. Aecidia

The aecidia are cupulate with a definite pseudoperidia ; the spores are binucleate and are formed in chains. The accidial initials are first formed deep down in the hypertrophied tissue. The hyphal cells of the plectenchyma that is formed are binucleate. The aecidiospores by infecting again the same plant, form secondary aecidia.

2. Pycnidia

Barclay (1891) observed only a few pycnidia in leaf sections, but was not able to identify them macroscopically. Ajrekar and Parendakar reported the absence of pycnidia in the material collected by them in Bombay. The writer was able to make them out macroscopically on flower buds, leaves, stem and fruits

by their glistening orange-yellow colour, when seen by reflected light.
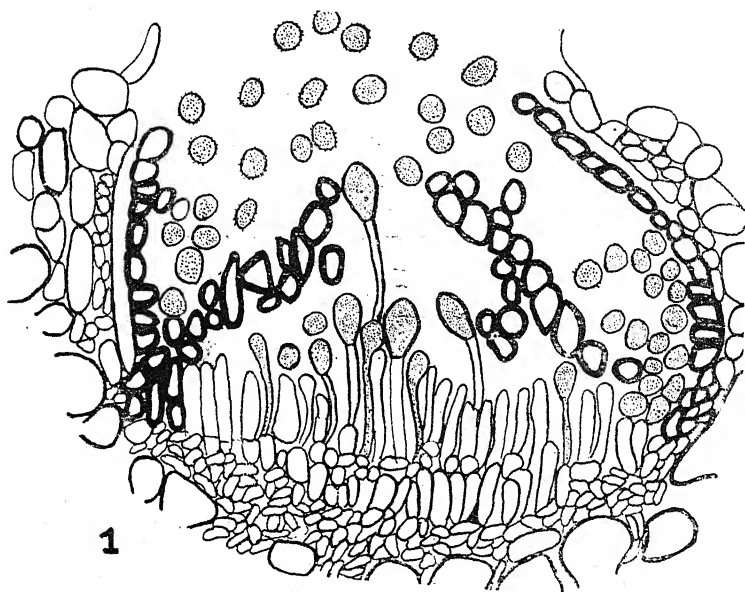
1. Development of teleutospores within the aecidial cup. X 360.

2. Development of teleutospores from the basal cells of the pycnidium X 300.

The pycnidia are sunken, amphigenis, and possess a definite ostiole. They are borne on binucleate mycelia, unlike as in other rusts. The basal cells of the pycnidia below the pycnidiosporophores are also binucleate, which can be made out in young pycnidia. Fusions between pycnidiospores and the hyphae at the ostiole were made out in microtome sections similar to the cases reported by Buller (1938) in *Puccinia graminis*.
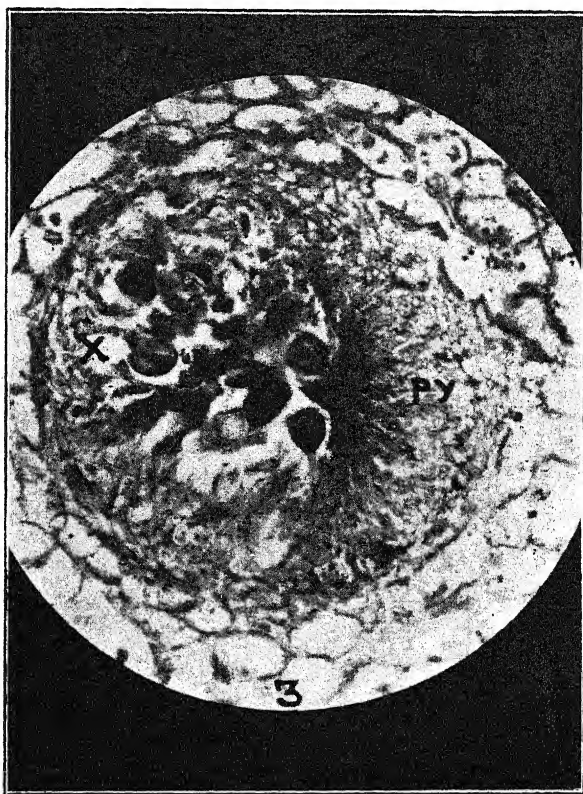
3. Photomicrograph of the development of teleutospores from the basal cells of pycnidium. Teleutospores are present in the region marked "X", X 360.

Occurrence of other spore forms in pycnidia

In many of the sections through pycnidia an unusual phenomenon was noticed. From the basal part of the mature pycnocarp aecidiospores and teleutospores were seen to develop. Early stages in the development of teleutospores within the pycnidia can be made out in Fig. 2 and 3. Owing to the transient nature of the pycnidia, only a few such cases have been met with. It is considered that this development is made possible on account of

the binucleate condition of the basal cells of the pycnidium, obviating diplodization.
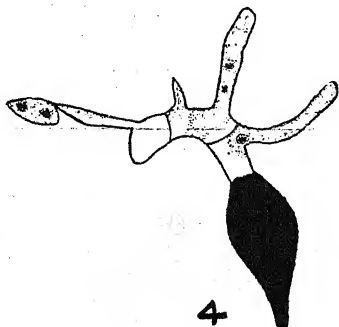
4. Germination of teleutospore showing binucleate sporidium. X 600.

3. Telia

Teleutospores are pear shaped, one celled, with a persistent stalk. They are always developed from the base of the old aecidial cups (Fig. 1). After the aecidiospores are shed, the teleutospore initials make their appearance. A similar case has been reported in *Uromyces alpestris* by Transzchel; but the course of development is different in *Uromyces Hobsoni.*
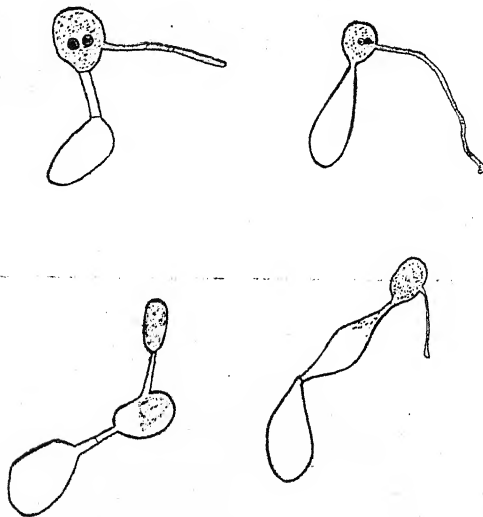
5. Development of secondary and tertiary sporidia. X 820.

Germination of Teleutospores

Teleutospores germinate without a rest period as indicated by the ready germination of fresh spores. The spore germinates and forms four to five cells in the promycelium. From each of these promycelial cells a sterigma is developed. The basidiospores are binucleate (Fig. 4). Development of secondary and tertiary sporidia were observed in many cases. (Fig. 5).

A detailed description of the life history of *Uromyces Hobsoni*, will appear in a separate paper. The writer wishes to acknowledge his indebtedness to Dr. M. A. Sampathkumaran, M. A., Ph. D., Professor of Botany, Central College, Bangalore, and to Mr. M. J. Narasimhan, Mycologist, Dept. of Agriculture, for helpful guidance and criticisms in the course of this investigation.

Literature Cited

AJREKAR, S. L. and Parandekar S. A. 1931.—Observations on the life history of the rust fungus *Uromyces* species on *Jasminum malabaricum*, and its relation to U. Hobsoni. Vize. (U. Cunninghamianus Barc.) JOURN. Ind. Bot. Soc. Vol. 10, 1931 ; P. 195-204, Pl—2.

BARCLAY, A. 1891.—On the life history of a remarkable Uredinae on *Jasminum grandiflorum* (*U. Cunninghamianus* nov. sp.) TRANS. Linn. Soc. (Bot.) Vol. 3, P: 141-151 Pl. 2.

BULLER, A. H. R. 1938. Fusions between flexuous hyphae and pycnidiospores in *Puccinia graminis*. Nature, Jan. 1st. 1938, P. 33-34.
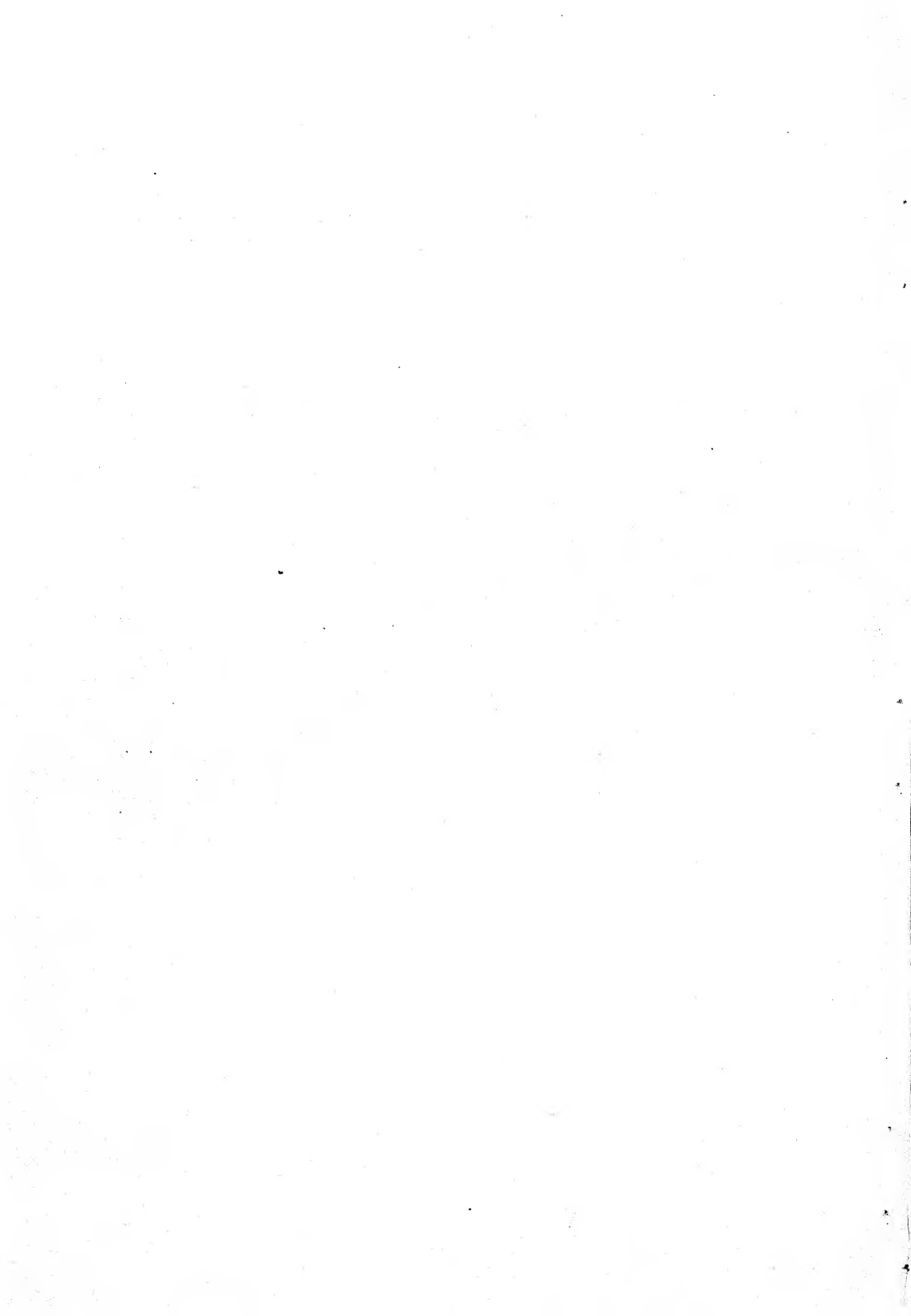
CYTOLOGICAL STUDIES OF CERTAIN MEMBERS OF THE FAMILY SAPROLEGNIA-CEAE—PART I.

BY

M. S. Murdia, M. Sc.

Research Scholar

Department of Botany, University of Allahabad

Introduction

During the close of the last century and in the beginning of the present one, the family *Saprolegniaceae* drew the attention of a number of workers specially from the point of view of sexual reproduction. Hartog (5) pronounced that there was no true fertilization occurring in this family while Trow (9) with his carefully controlled technique went to establish that true fertilization was undoubtedly of occurrence in some of its genera. Since that time a number of genera have been investigated and Trow's results have been confirmed with regard to the presence of true fertilization in this family. Besides this, various other workers have studied a number of its genera from the cytological and physiological point of view and results of considerable interest have been obtained. The present writer took up this study in September, 1937 in order to supplement the present knowledge with cytological and physiological observations that appear intimately related to the lifehistory of some of the genera of this family.

It was thought advisable to begin with the cytological study of the mycelium. This paper thus embodies the first part of the work in hand, and the details of sexual reproduction and other necessary observation will follow in a subsequent paper.

Material

As a preliminary to this work it was decided to collect as many local species as possible. A number of samples of water were brought from different localities near about Allahabad and from these only two watermoulds were isolated, of which one was *Achlya dubia* Coker, and the other was *Aphanomyces* sp. Following method was

adopted for their isolation. Killed ants, which served as baits, were left in the samples of water for 24 hours and later transferred to sterile distilled water after being washed with slightly acidulated water. In a couple of days fungal hyphae radiated from these ants and later formed zoosporangia which discharged clusters of zoospores. A cluster of the latter was picked up by means of a bent capillary tube and transferred to an agar medium in petridishes. The zoospores germinate here and form a zone of mycelium. These cultures so obtained were further purified from any bacterial contamination by repeated subculturing on fresh agar plates.

The genera were identified with the help of Coker's monograph on *Saprolegniaceae* (1) on the basis of the structure of zoosporangia and behaviour of zoospores. Species of *Achlya* could also be identified as it formed the sexual organs while that of *Aphanomyces* remains as yet to be identified, as it has failed to form sexual organs inspite of being subjected to a number of nutritional and environmental conditions. For the work to be in progress a number of forms were obtained from Centraalbureau von Schimmelcultures, Baarn, Holland, which have, as far as the present writer could gather, received little cytological investigations. These are *Pythiopsis intermedia* Coker, *Aphanomyces cladogamus* Drechs., *Aphanomyces camptostylus* Drechs. and *Thraustotheca clavata* (deBary) Humphrey.

Observations on living material

While the mycelium of *A. dubia* and *T. clavata*, with their relatively broad hyphae, afforded a favourable material for the observation of cytoplasmic inclusions, that of *P. intermedia* and species of *Aphanomyces* was much less favourable due to the relative thinness of their hyphae.

Fat particles :—

Under the high power of the microscope there are observed in the mycelium, which is hyaline in all cases, a large number of minute round bodies (fig. 6) rapidly moving in the cytoplasm. These take a reddish orange tint with Soudan III become brownish when treated with a 2% solution of osmiumtetraoxide and dissolve readily in chloroform, ether and benzine. These reactions go to show that they are of a fatty nature. These fat particles vary in size and number with the age of the hyphae as also from one hypha to the other. In the younger tips they are not so numerous, while in some of the older hyphae they are so much crowded that no other cytoplasmic inclusions, except them, are visible. In some of the broader hyphal tips of *A. dubia* and *T. clavata* they were found to be much crowded but such hyphal tips later gave rise to zoosporangia.

Mitochondria :—

With careful observation under high magnification and with experience, they are seen in the cytoplasm without any staining. In *A. dubia* (fig. 6) and *T. clavata* they are filamentous, extremely delicate and of varying length lying for the most part parallel to the long axis of the hyphae. In *P. intermedia* besides such filamentous ones, granular mitochondria, have also been observed, while in species of *Aphanomyces* both types are equally common. The mitochondria, whether filamentous or granular have nearly the same refraction as that of the cytoplasm and move very sluggishly in it. These two characters are helpful in distinguishing the granular mitochondria from the fat particles.

Nuclei :—

Due to the favourable width of hyphae in *A. dubia* and *T. clavata*, nuclei in living condition could be observed in them with a careful search. These have a circular and in the majority of cases an elliptical outline with a refracting spot in the centre representing the nucleolus Nuclei in living condition could not be observed in others.

Vacuoles :—

At the hyphal tips in all cases, small round or ellipsoidal vacuoles were seen but they were not distinct due to their refracting power being nearly the same as that of the cytoplasm surrounding them.

Supravital staining

Mitochondria :—

Janus green Höcht B. and dahlia violet dissolved in Ringer's solution were employed to stain mitochondria in living condition. Both these dyes are very toxic and so very minute doses have to be used. Both of them do not stain the mitochondria so quickly as the vacuolar staining dyes (mentioned below) do the vacuoles, and the mycelium has to be kept in the solution for about 10-12 minutes before the mitochondria take up the stain. As a matter of fact, due to their toxicity, they stain the mitochondria in phases preceding the death of the hypha. With janus green they take up a sky blue colour while dahlia violet gives them a light violet tinge. Their structure after being stained is nearly the same as described above when no stain was employed, but a majority of them appear in the process of fragmentation and vesiculisation (fig. 7). A curious phenomenon observed was that janus green which at first is taken as a bluish stain by the hyphae is later (after about 12-15 minutes) turned to pink. This has. been accounted as due to the formation of a reduction product of the dye by the activity of the cytoplasm. Saksena (6, p. 64) has also observed this reduction of janus green in four species of *Pythium.*

Nuclei :—

No satisfactory vital dye was found that could stain the nuclei in living condition. However a dilute solution of nile blue in distilled water stained them in *A. dubia* and *T. clavata*. Dahlia violet in dilute solutions also stained them to a certain extent.

Vacuoles :—

Vital staining of vacuoles was tried with a number of vital dyes viz. neutral red, cresyl blue, nile blue, methylene blue and toluidine blue. They were made into solution with Ringer's solution and different concentrations were used. In all cases the vacuoles absorbed the dyes quickly and became coloured. With neutral red the vacuoles took up a reddish orange tint, light or deep according to the concentration used, while with other dyes the colour varied from blue and bluish violet to violet. Of all the dyes neutral red was decidedly unexcelled as it penetrated the vacuoles quickest and was least toxic thereby allowing a much better observation. In all cases the tips of young hyphae formed an important and interesting place for observation. After being treated for about a minute with any of the vacuolar vital dyes, the vacuolar system can be seen in its initial stage at these tips. This presented itself in some tips in the form of a reticulum (fig. 1) formed by thin elongated vacuoles which by irregularly fusing with one another gave the appearance of a reticulum. In the older portion of the same hypha this reticulum becomes less and less evident due to the increase in width of these vacuolar canalicules and their more frequent fusions till finally in the still older portion a continuous vacuolar canal results. In other tips small round vacuoles followed by ellipsoidal ones (fig. 2) were observed and the latter by fusions form a continuous vacuolar canal in the older portion of the same hypha. The colour of the young vacuoles at the tips, after treatment with the vital dyes is deeper than that of the vacuolar canal in the older portion, which is due to the gradual dilution of the vacuolar sap from the tip to the older portion.

Intravacuolar corpuscles:—

These corpuscles make their appearance in the vacuoles when the latter are treated with a dilute solution of any of the vacuolar vital dyes. Their origin in the vacuoles is an interesting phenomenon to be studied. A single hypha is kept under observation under the microscope and a weak neutral red solution is passed on from one side of the coverslip and drawn at the other by a strip of filter paper. As soon as the hypha gets bathed in the solution, the vacuolar canal gets coloured while in its interior very minute dark particles become visible in brisk Brownian movements on account of the adsorption of the dye (fig. 3). In less than a minute their movements get retarded and they become more visible due to an increase in their size (fig. 4).

Later these become much bigger and fewer, show little movements, and come to lie as deep red intravacuolar corpuscles in the vacuoles (fig. 5). Their increase in size and reduction in number is due to the fusion of one another as they collide while they are smaller and in brisk movements. Their appearance under the action of the dye is due to a disturbance in the colloidal state of the vacuolar sap resulting in the precipitation of its colloidal particles.

A second category of intravacuolar corpuscles observed in *A. dubia* and *T. clavata* was rather interesting. These are round and of varying size up to 4 ʋ. They are observable in the hyphae even without any staining (fig. 6). They are slightly more refracting than the cytoplasm and are found singly or in groups of two, three or more. They take up the vacuolar vital dyes quickly and stain themselves deeper than the vacuole itself, although lighter than the intravacuolar corpuscles described above. They are not stained with Soudan III or scarlet red and are insoluble in absolute alcohol, formol, 10% potassium or sodium hydroxide, ether, benzine and xylol. Nothing can be said at this stage regarding their origin, nature and function.

Tests were also made for *metachromatic corpuscles* reported in the vacuoles of *Endomyces magnussi, Penicillium glaucum* and *Saccharomyces Ludwigi* by Guillermond (2). The test consists in at first fixing the mycelium in either absolute alcohol or formol for an hour and then either (a) staining in a 1% acqueous solution of cresyl blue for a minute, washing rapidly in water and mounting in glycerine when the *metachromatic corpuscles* take a red stain or (b) staining in a 1% acqueous solution of methylene blue for a minute and mounting in water acidified with sulphuric acid when only the *metachromatic corpuscles* remain coloured. None of the forms under investigation gave any positive test for corpuscles of this nature.

A mixture of neutral red and janus green was employed in each case to stain the vacuolar and mitochondrial systems simultaneously. It was observed that the two dyes were taken up specifically by the two systems respectively, the vacuoles colouring themselves orange red while the mitochondria assume a bluish appearance.

Intravital staining

In order to stain the vacuolar system intravitally the fungi were grown in 1% bacto-peptone to which vacuolar vital dyes were added in concentrations ranging from 0.25 to 10 mgs. %. From the point of view of toxicity of these dyes neutral red was found to be the least toxic followed by cresyl blue, toluidine blue, methylene blue and nile blue, the last being the most toxic. As regards the colouration of the vacuolar system, most satisfactory results were obtained with neutral red as the

other dyes fall short of this due to their greater toxicity and higher oxido-reduction potential as has also been mentioned by Guillermond (4, p. 296-97). The best concentration of neutral red for the vacuolar study was from 2 to 4 mgs. % for *A. dubia* and near about 2 mgs. % in others. As the concentration was increased, growth was more and more retarded as was also observed by Saksena (6,p.30) and Guillermond (3), and with the following doses of neutral red there was no visible growth of the fungi.

T. clavata	.. 5 mgs. %
A. dubia	.. 9 mgs. %
Aphanomyces camptostylus	.. 5 mgs. %
Aphanomyces cladogamus ..	.. 6 mgs. %

A microspic examination of the hyphae stained intravitally with neutral red revealed the same phenomena as were observed with supravital staining. In the living portion of the mycelium, the vacuolar system in its initial stages (as described above) and the continuous vacuolar canal together with the intravacuolar corpuscles were observed. In the older portion of the mycelium, where the majority of the hyphae were dead, the stain was taken up by the cytoplasm in general and the colour thereof was pink and not orange red as in the living hyphae.

Observations on fixed material

Mitochondria :—

The fungi were grown in 1% bacto-peptone for 24-48 hours after which they were fixed in the following mitochondrial fixatives and treated subsequently as indicated below :—

(1) Regaud's liquid;

Potassium dichromate 3%	..	80 c.
Neutral formol	..	20 c.

Fixed for 4 days, changing the liquid after every 24 hours. Post-chromised in 3% potassium dichromate for a week. Washed in running water for 24 hours.

(2) Regaud-Tupa's liquid;

Potassium dichromate ..	..	80 c. c.
Neutral formol	..	20 c. c.
Uranium nitrate ..	..	1 g m.

Fixed for 48 hours at 10-12° C changing the liquid after every 12 hours. Washed in distilled water for 8 hours.

(3) Helly's liquid;

A.	Potassium dichromate ..	..	2.5 gms.
	Mercuric chloride ..	..	5.0 gms.
	Distilled water ..	..	100 c. c.

    B.   Neutral formol

9 c. c. of A are mixed with 1 c. c. of B at the time of fixation.

Fixed for 8 hours. Post-chromised for 48 hours at 40° C. Washed in running water for 24 hours.

(4) Sublime-formol;

Saturated solution of mercuric chloride ..	..	..	80 c. c.
Neutral formol ..	..		20 c. c.

Fixed for 24 hours. Post-chromised in 5% potassium dichromate for 2 days. Washed in running water for 24 hours.

(5) Liquid of Lenhossek;

Mercuric chloride 6% ..	..	80 c. c.
Absolute alcohol ..	..	20 c. c.
Acetic acid ..	..	3 c. c.

Fixed for 24 hours. Washed in running water for 24 hours.

In each case the washing was followed by staining of the mycelium *en masse* in iron-alum haematoxylin and its dehydration. Finally it was mounted in Canada balsam.

Mycelium was also embeded in each case in paraffin and microtome sections 5μ thick were cut to study the mitochondria. The slides were stained in iron-alum haematoxylin. This appeared rather a lengthy and laborious process and moreover did not show any greater advantage over the *en masse* mounting method.

The most satisfactory of all the fixatives was found to be Helly's liquid though Sublime-formol also gave sometimes equally good preparations. Regaud's and Regaud-Tupa's liquids gave preparations of an inferior quality. Lenhossek's liquid was tried mainly to study the action of acetic acid and alcohol on the mitochondria.

In all good preparations the mitochondria are stained black while the cytoplasm remains nearly colourless. The structure of the mitochondria is seen much better in these

fixed preparations than in the living condition. As observed above, there are only filamentous forms of mitochondria in *A. dubia* and *T. clavata* (figs. 8 and 9) while in others (figs. 11, 12 and 13) both filamentous and granular forms can be observed in these preparations. At places the filamentous ones are seen in the process of transverse fragmentation into two daughter mitochondria. Occasionally portions of hyphae are met with in these preparations presenting a honey-combed structure, which is due to the vesiculisation of all their mitochondria. Most of the granular mitochondria observed in *P. intermedia* and species of *Aphanomyces* result, in the opinion of the present writer, from the fragmentation of the existing filamentous types and the subsequent rounding off of these fragments, preparatory to their being finally vesiculised.

With Liquid of Lenhossek which contained 3% acetic acid and 20% absolute alcohol, mitochondria were found to be preserved in all cases, though not so distinctly as with other fixatives. Acetic acid and alcohol have been found to destroy mitochondria in animal cells as well as in higher plants and their preservation in these cases speaks of their different chemical nature. In *T. clavata* all the mitochondria tend to become vesiculised when this fixative is employed (fig. 10).

Nuclei :—

A number of nuclear fixatives (6, p. 11) viz. that of Bouin, Claussen, Trow, Merkel with acetic acid modified by Smith (8). and Saksena have been so far tried. Saksena's osmic fixative has given fairly good results in the two species of Aphanomyces while Merkel's with acetic acid has been quite successful for *A. dubia* and *T. clavata*. Iron-alum haematoxylin was used in all cases for staining the nuclei. The structure of the nuclei as observed in some good preparations was as described by Saksena (7) for the genus *Pythium*. The main portion of the nucleus consists of a central body which takes a dark stain. Surrounding this, is a layer of nucleohyaloplasm which is bounded externally by a distinct nuclear membrane. The central body is seen connected to the nuclear membrane by fine threads and on the inner side of the nuclear membrane minute dark mounds are observable which are said to represent the chromatin material of the nucleus. No mitotic division of the nucleus has so far been observed in the vegetative hyphae though amitotic division as described by Smith (8) has been found to occur in them.

Summary

1. An account of the cytological studies of the vegetative mycelium of the following five members of the family *Saprolegniaceae* has been given :— *Achlya dubia, Pythiopsis intermedia, Aphanomyces camptostylus, Aphanomyces cladogamus* and *Thraustotheca clavata*.

2. Observations on the living material were made both with and without vital staining. When no stain was used, there were observed in the hyphae fat particles, mitochondria which were filamentous in *A. dubia* and *T. clavata* and both filamentous and granular in others, nuclei in *A. dubia* and *T. clavat* and vacuoles at the hyphal tips of all the forms. Mitochondria were stained supravitally with janus green Höcht B. and dahlia violet but both of them were very toxic and stained the mitochondria in phases preceding the death of the hyphae. A number of vacuolar vital dyes were employed to colour the vacuolar system both supravitally and intravitally. Neutral red was found to be the least toxic and a most satisfactory dye for this purpose. No *metachromatic corpuscles* have been found in these fungi.

3. In each case the mycelium was fixed in a number of mitochondrical fixatives. Helly's liquid was found to be most satisfactory. The structure of the mitochondria in fixed preparations was as observed in the living condition of the hyphae. Acetic acid and alcohol did not destroy the mitochondria in any of these fungi.

4. A number of nuclear fixatives were used to fix the nuclei. Iron-alum haematoxylin was employed to stain them. Saksena's osmic fixative gave good results in the two species of *Aphanomyces* while Merkel's with acetic acid proved quite successful for *A. dubia* and *T. clavata*. The structure of the nuclei was observed and found to correspond to that described by Saksena for the genus *Pythium*. No mitotic division was observed. Amitotic division has been occasionally found.

In the end it is my pleasant duty to express my gratitude to Prof. J. H. Mitter who has given me all the laboratory facilities, and my indebtedness to Dr. R. K. Saksena, under whose supervision this work has been carried, for his continuous interest and encouragement.

Literature cited

1. COKER, W. C.—(1923). *Saprolegniaceae.*

2. GUILLERMOND, A.—(1929). The recent development of our idea of the vacuome of plant cells. American Jour. Bot. Vol. XVI.

3. GUILLERMOND, A.—(1929). Sur le development d'un *Saprolegnia* en millieu additionne de colorants vitaux et coloration vitale de son vacuome, pendant son development. C. R. Ac. Sc. Vol. CLXXXVIII.

4. GUILLERMOND, A. MANGENOT, G. AND PLANTEFOL, L.—(1933). Traite de cytologie vegetale. Librairie E. Le Francois, Paris.

5. HARTOG, M. M.—(1899). The alleged fertilization in *Saprolegniaceae* Ann. Bot. Vol. XIII.

6. SAKSENA, R. K.—(1935).   Recherches physiologique et cytologique sur quelque especees du genere *Pythium*. Librairie.   Generale de l' enseignement.   Paris.

7. SAKSENA, R. K.—(1936).   Structure of the nucleus in the genus *Pythium*.   Jour. Ind. Bot. Soc. Vol. XV.

8. SMITH, FRANCIS E. V.—(1923). On direct nuclear division in the vegetative mycelium of *Saprolegniaceae*. Ann. Bot. Vol. XXXVII.

9. TROW, A. H.—(1904).   On fertilization in *Saprolegniaceae* Ann. Bot. Vol. XVIII.

Explanation of plate XII

Abbreviations used :—

C=Cytoplasm; F=Fat particles; Ic_1=Intravacuolar corpuscles formed under the action of neutral red; Ic_2=Pre-existing intravacuolar corpuscle; M=Mitochondria; M_1=Mitochondrium in the process of being vesiculised; M_2=A fragmented mitochondrium M_3=Fragments of a mitochondrium which have become vesiculised; Mg=Granular mitochondria; N=Nucleus; R=Vesiculised mitochondrium; T=Tonoplasm; V=Vacuole; Vc=vacuolar canalicules.

Figs. 1-13. Hyphal tip of *Aphanomyces camptostylus* treated with neutral red solution, showing the initial vacuolar system in the form of a reticulum. ($\times$ 1600). Fig. 2. Hyphal tip of *Aphanomyces camptostylus* treated with neutral red solution, showing the initial vacuolar system in the form of round and ellipsoidal vacuoles ($\times$1600). Figs. 3, 4 and 5. A portion of a young filament of *A. dubia* sketched at three stages after being treated with neutral red. The figures show the gradual evolution of intravacuolar corpuscles in the vacuole. ($\times$ 1600). Fig. 6. A portion of a filament of *A. dubia* as seen in the living condition without being treated with any vital dye ($\times$ 1200). Fig. 7. A portion of a filament of *A. dubia* after being treated with dahlia violet for about 15 minutes. ($\times$ 1200). Fig. 8. A portion of a filament of *A. dubia* fixed in Sublime-formol showing filamentous mitochondria and nuclei. ($\times$ 1200). Fig 9. A portion of a filament of *T. clavata* showing filamentous mitochondria and nuclei. ($\times$ 1200). Fig. 10. A portion of a filament of *T. clavata* fixed in the Liquid of Lenhossek, showing the fragmentation and vesiculisation of its mitochondria ($\times$ 1200). Fig. 11. A portion of a filament of *P. intermedia* fixed in Helly's liquid showing only the filamentous mitochondria ($\times$ 1600). Fig. 12. A portion of a filament of *P. intermedia* fixed in Helly's liquid showing granular mitochondria in large numbers ($\times$ 1600). Fig. 13. A portion of a filament of *A. cladogamus* fixed in Helly's liquid showing both granular and filamentous mitochondria ($\times$ 1600).
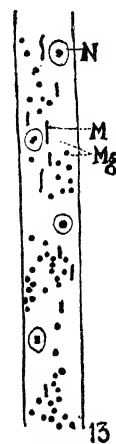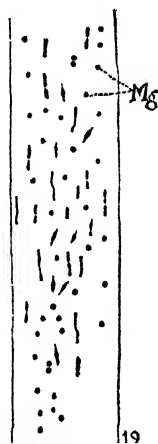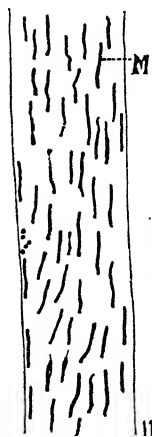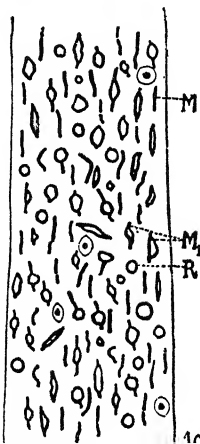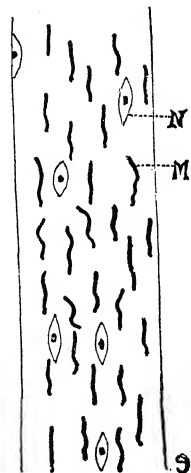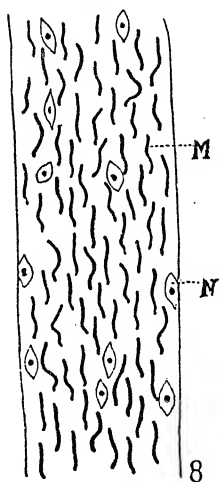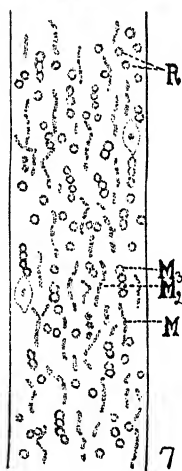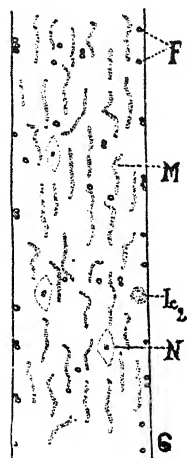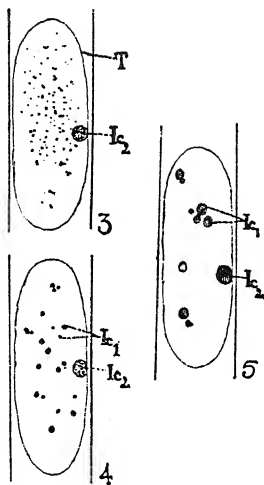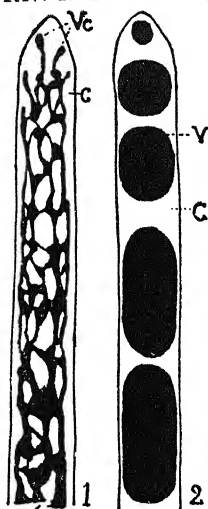
ON TWO SPECIES OF *ANTHOCEROS* FROM CHINA

BY

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For the material which forms the basis of this note the writer is indebted to Professor H. H. Chung, Wu-Han University Wuchang, Hupeh, China, to whom he wishes to express his thanks.

Anthoceros fulvisporus Stephani 1916

Description

Monoecious. In pale green patches, blackened when dry. Thallus 10-15 × 3-5*, nearly flat, divided into obovate lobes, with the margin entire or wavy ; surface cells 0·035 — 0·07×0·025 — 0·035, transverse section 5-6 cells high in the middle, without lacunae. Involucre sometimes geminate, 2·5 — 3·5 long and 0·5-1·0 broad, cylindrical, slightly narrowing towards apex, mouth repand. Capsule 14-25 long and 0·25 — 0·35 broad, dark brown ; stomata 0·06 — 0·07×0·025 — 0·03. Spores 0·04 — 0·045 greenish yellow, very lightly granular papillate ; pseudoelators 1-6 celled, variously shaped, pale brown.

Collector. S. C. Sun.

Locality. Lokiashan, Wuchang, Hupeh Prov. China.

University College, Rangoon Museum No. 1458.

From the above comparative chart of the pale spored species of *Anthoceros* Linn. 1753 without lucunae in the thallus the following species agree with the form described in having approximately the same type of spore surface: *A. argentinus* Jack et Stephani 1895, *A. brotheri* Stephani 1916, *A. brunnthaleri* Stephani 1916, *A. carolinianus* Michaux 1803, *A. colensoi* Mitten 1885, *A. dichotomus* Raddi 1808, *A. esquirolii* Stephani 1923, *A. fimbriatus* Gottsche 1864, *A. fulvisporus* Stephani 1916, *A. gualaquizana* Stephani 1916, *A. laevis* Linn. 1753, *A. leratii* Stephani 1916, *A. mildbraedii* Stephani 1923, *A. nordenskjoldii*

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* All measurements in millimeters.
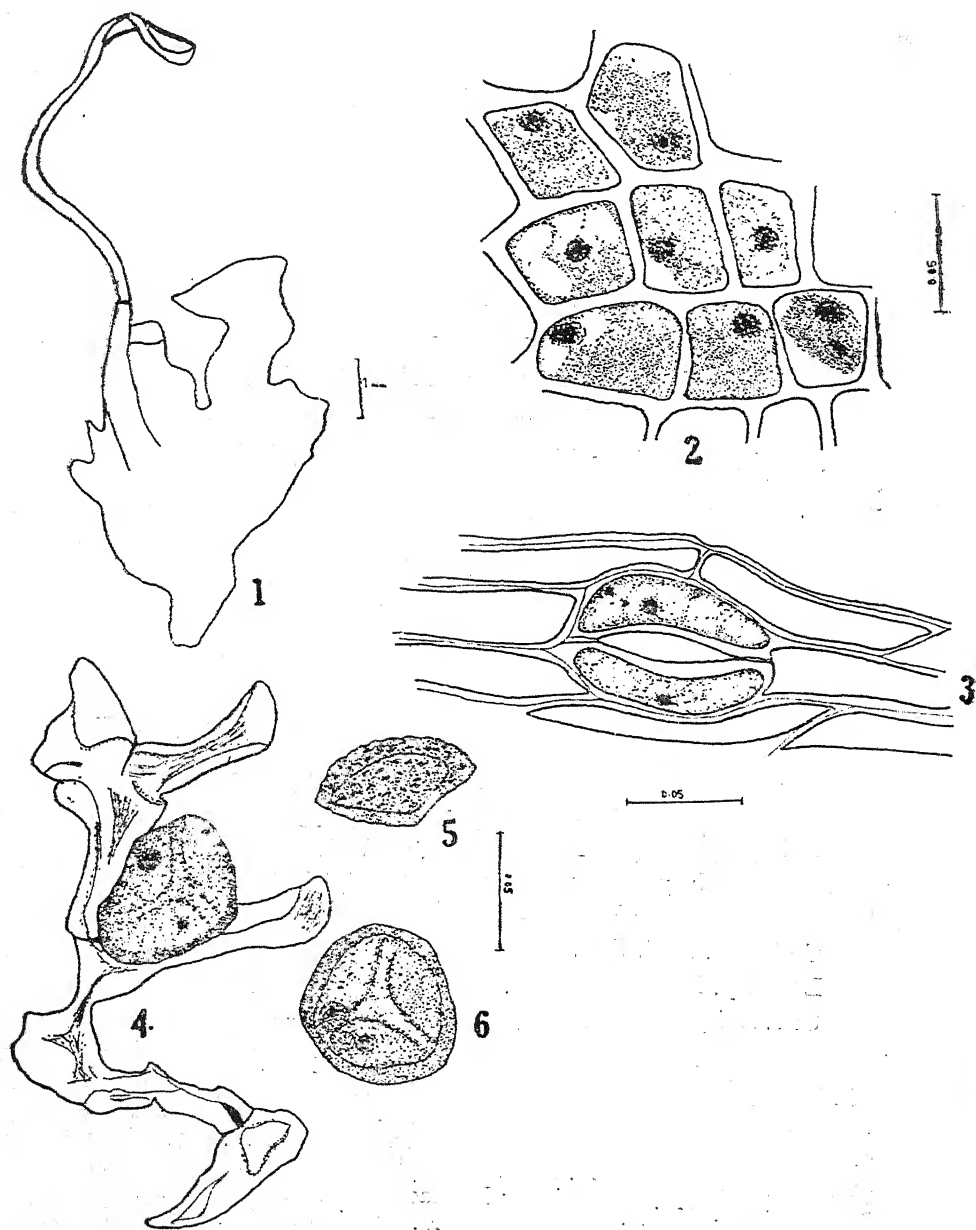
Figs. 1-6.—*Anthoceros fulvisporus* Stephani. Fig. 1 Young Plant; Fig. 2 Dorsal epidermis cell; Fig. 3 Stoma; Fig. 4 Psuedoelator with a spore; Fig. 5-6 spores.

Chart showing differences between those species of Anthoceros Linn. 1753 with solid thallus and pale spores

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
argentinus Jack et Stephani 1895.	Monoecious	10	2	15	·035 yellowish green papillate.	Argentina. !
arsenii Stephani 1923	Monoecious	15	4	25	·036 minute, asper,	Mexico.
atlanticus Stephani 1916	Monoecious	15	4	40	·036 subhispid.	Africa : Teneriffa. !
autoicus Stephani 1916	Monoecious	20	4	35	·036 levis.	Asia : Nova Caledonia.
brevicapsulus Stephani 1916	Monoecious	15	3	20	·036 asper.	Cuba.
bolanderi Stephani 1916	Monoecious	5	2	30	·036 levis.	Europa.
brotheri Stephani 1916	Monoecious	20	2	15	·036 palegreen, papillate.	Australia : Upper Owens River.
brunnthaleri Stephani 1916	Monoecious	15	2	30	·045 granular.	Africa : Usambara, Amanii.
butleri Stephani 1916	Monoecious	12	5	20	·036 levis.	Himalaya.
caroliniamus Michaux 1803	Monoecious	30	5	50	·045 green, papillate.	America.
chiloensis Stephani 1916	Monoecious	25 × 5	4	65	·045 asper.	America australis. Insula Chiloé.
colensoi Mitten 1877	Dioecious	40 × 3	3	40	·036 muricate.	Australia : Nova Zelandia.
communis Stephani 1897	Monoecious	15 × 5	3	20	·036 asper.	America.
coriaceus Stephani 1916	Dioecious	10	2	15	·045 asper.	Australia : Nova Zelandia.
dendroceroides Stephani 1916	Dioecious	20	2	20	·045 asper.	Chile.
dichotomus Raddi 1808	Dioecious	20 × 5	5	40	·036 highly papillate.	Europa : Italia ; Hispania.

Chart showing differences between those species of Anthoceros Linn. 1753 with solid thallus and pale spores—contd.

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
esquirolii Stephani 1923	Monoecious	7 × 3	2	30	·036 green muricate.	China.
fimbriatus Gottsche 1864	Monoecious	20	3	25	·036 papillate.	America : Nova Granada.
fulvisporus Stephani 1916	Monoecious	20	4	35	·036 yellow, papillate.	Africa : Kilimands-charo.
glaziovii Stephani 1916	Dioecious	··	2	30	·036 green, levis.	Brasilia.
gualaquizana Stephani 1916	Monoecious	15	3	40	·027 minute granular.	America.
jackii Stephani 1923	Monoecious	15 × 5	3	30	·036 minute asper.	Himalaya : Mussuri.
kuntzeanus Stephani 1916	Monoecious	20 × 2	4	35	·036 asper.	America : Portorico.
laevis Linn. 1753	Monoecious	15	2	30	·036 papillate.	Europa ; America ; India.
leratii Stephani 1916	Monoecious	20	3	35	·04 papillate.	Nova Caledonia.
luzonensis Stephani 1916	Monoecious	12×3	5	40	·036 hispid.	Philippinae Insula.
maritimus Stephani 1916	Dioecious	··	··	··	··	Falkland Islands.
mildraedii Stephani 1923	Monoecious	12×3	5	30	·036 papillate.	Africa : Camerun.
multicapsulus Stephani 1916	Monoecious	10	3	50	·045 asper.	Australia.
nordenskjoldii Stephani 1923	Monoecious	15×5	3	25	·045 green muricate.	Japonica.
parvifrons Stephani 1916	Monoecious	5	2	15	·036 levis.	Africa : Usambara.
parvus Stephani 1923	Monoecious	10×3—5	Short	15	·036 muricate.	Mexico.

Chart showing differences between those species of Anthoceros Linn. 1753 with solid thallus and dark spores

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
planus Stephani 1893	Monoecious	..	4	80	·036 asper.	Brasilia.
polyandrus Stephani 1916	Dioecious	20	2	20	·027 leves.	Java.
psuedocostus Stephani 1916	Monoecious	8×3	4	30	·036 papillate.	Madagascar.
pusillus Stephani 1916	Monoecious	5×2	2	25	·027 asper.	Hawai.
radicellosus Stephani 1916	Dioecious	10×3	3	20	·036 asper.	Japonia.
sylvaticus Stephani 1916	Monoecious	10×3	3	35	·036 asper.	Java.
speciosus Jack 1923	Monoecious	30×10	4	40	·045 muricate.	Chile.
squamuligerus Spruce 1885	Dioecious	25	5	30	·04 leves.	Ecuador: Pichincha.
sumatranus Stephani 1916	Monoecious	15	2	20	·036 leves.	Sumatra.
tenerrimus Stephani 1916	Monoecious	15	5	30	·036 leves.	Brasilia.
tenuissimus Stephani 1893	Monoecious	30×5	10	50	·036 green papillate.	Africa.
tuberosus Taylor 1916	Monoecious	12	5	30	·036 minute asper.	Australia.
undulatus Stephani 1916	Monoecious	15	5	50	·036 leves.	Brasilia.
usambarensis Stephani 1916	Monoecious	15×5	5	40	·036 granular.	Africa.
validus Stephani 1916	Monoecious	10	3	50	·036 papillate.	Java.
wettstenii Stephani 1916	Monoecious	..	3	25	·036 leves.	Brasilia: Sao Paulo.

Stephani 1923, *A. parvus* Stephani 1923, *A. pseudocostus* Stephani 1916, *A. speciosus* Jack 1923, *A. tenuissimus* Stephani 1893, *A. usambarensis* Stephani 1916 and *A. validus* Stephani 1916. The present form differs from *A. carolinianus* Michaux 1803, *A. colensoi* Mitten 1855, *A. gualaquizana* Stephani 1916, *A. speciosus* Jack 1923, *A. tenuissimus* Stephani 1893, *A. usambarensis* Stephani 1916 and *A. validus* Stephani 1916 by the shorter capsule; from *A. brunnthaleri* Stephani 1916, *A. fimbriatus* Gottsche 1864, *A. leratii* Stephani 1916, *A. mild-braedii* Stephani 1923, *A. parvus* Stephani 1923 and *A. pseudocostus* Stephani 1916 by the greenish yellow colour of the spore; from *A. argentinus* Jack et Stephani 1895, *A. dichotomus* Raddi 1808, and *A. laevis* Linn. 1753 by the shape and other characters of the thallus, *A. brotheri* Stephani 1916, *A. esquirotii* Stephani 1923, *A. fulvisporus* Stephani 1916, *A. nordenskjoldii* Stephani 1923 and the above described form differ from each other in minor characters—which are not sufficiently distinct to be of specific value. The writer therefore is of the opinion that these may be considered as a single species—*A. fulvisporus* Stephani 1916.

The writer has been unable to consult material of these forms and is therefore unable to state whether differences exist which would supplement the already existing descriptions. Judging from the characters so far given however he is of the opinion that there is only one species, *A. fulvisporus* and the other three listed are environment forms without specific validity.

Anthoceros Chungii sp. nov.

Description

Dioecious. In dark green patches. Thallus 4-10x3-5, depressed in the centre, margin ascending, divided into numerous, narrow linear or more or less cuneate lobes; surface cells 0.035-0.045x 0.01-0.025, transverse section of middle of the thallus 10-16 cells high, lacunae large. Involucre 2-4.5 long 0.3-0.5 broad, oval sharply narrowed towards the apex, mouth lobulate. Capsule 15-45 long and 0.2-0.3 broad,; stomata 0.05-0.07x 0.02-0.03 (guard cells unequal. Spores 0.03-0.04 light brown granular papillate. Pseudoelators brown, of 2-6 cells.

Antheridia in groups of 2-4.

Collector. H.H.Chung.

Locality. Kiangsi Prov. China.

University College, Rangoon Museum No. 1459

From the chart of cavernous species of *Anthoceros* Linn. 1753 with dark spores it will be seen that the following species approach the described form in possessing approximately the same type of spore surface: *A. angustus* Stephani 1916, *A.chevalieri* Stephani 1923, *A. cristatus* Stephani 1916, *A. faurianus* Stephani 1916, *A. fertilis* Stephani 1916,

Figs. 7-11.—*Anthoceros Chungii* sp. nov. Fig. 7 Female Plant; Fig. 8 A lobe of the thallus; Fig. 9 Dorsal epidermis cells; Fig. 10 Stoma Fig. 11 Spore.

Chart showing differences between those species of Anthoceros Linn. 1753 with cavernous thallus and dark spores

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
angustus Stephani 1916 ..	Monoecious	20×3.	3	20	·036 dark, muricate.	Asia : Himalaya, Kurseong.
apiahynus Stephani 1916 ..	Monoecious	20	10	25	·036 dark, hispidulous.	America : Brasilia Apiahy.
brunneus Stephani 1916 ..	Monoecious	10	3	25	·045, black, echinate.	Asia : Tonkin.
butneri Stephani 1916 ..	Monoecious	10×2	2	20	·045, black, hispid.	Africa : Togo, Bismarkburg.
caespiticus De Notaris 1839 ..	Monoecious	15	6	50	·045, dark, hispidulous	Europa meridionalis : Hibernia, Caucasus.
cataractarum Stephani 1916 ..	Monoecious	25×5	2	20	·036, black, hispid.	Asia : Himalaya.
cavernosus Stephani 1916 ..	Monoecious	20	6	30	·036, black, hispid.	America : Martinique.
chambensis Kashyap 1917 ..	Dioecious	30	2·5	25	·04—·048 opaque.	Asia : Chamba Valley ; Sialkot.
chevalieri Stephani 1923 ..	Dioecious	20	..	20	·045 dark brown, papillate.	Africa centralis.
constans Lindberg 1887	Monoecious	30	6	15	·054 black, hispid.	Portugal : Tenerifa.
crassifrons Stephani 1916 ..	..	20	..	..	..	Africa : Acores Insulae.
crispulus Douin 1905 ..	Monoecious	5	3	20	·045 black, hispid.	Gallia.
cristatus Stephani 1916 ..	Monoecious	5	1	25	·036 dark, papillate.	Africa : Ascension Island.
cucullatus Stephani 1916 ..	Dioecious	15	2	50	·036 darkbrown, asper.	America : Guadeloupe.
erectus Kashyap 1915 ..	Dioecious	10	5	30	·03—·04 black, granulose.	India.

Chart showing differences between those species of Anthoceros Linn. 1753 with cavernous thallus and dark spores—contd.

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
faurianus Stephani 1916	Dioecious	10	3	35	·036 black, asper.	Asia: Japonia, Mokuto.
ferdinandi Taylor 1864	Dioecious	10	6	60	·045 black, echinate.	Australia: Port Denison.
fertilis Stephani 1916	Monoecious	5	1	15	·045 dark, long papillae.	Australia: Queensland.
fissus Stephani 1916	Monoecious	5	1	20	·045, black, hispid.	Asia: Nova Caledonia.
formosae Stephani 1916	Monoecious	20	3	6	·036, black, hispid.	Asia: Insula Formosa.
fragilis Stephani 1916	Monoecious	20	2	20	·036, hispidulous.	Australia: Queensland.
fuscus Stephani 1916	Monoecious	10	3	25	·045 dark, hispid.	Anam: (India Orientalis).
fusiformis Austin 1877	Monoecious	8	6	50	·045 dark, dense papillae.	America.
gottschei Stephani 1923	Monoecious	20×5	5	25	·045 light brown hispid.	Guadeloupe.
granulatus Gottsche 1864	Monoecious	15	4	40	·045 black, echinate.	America: Mexico.
grosse-involucratus Stephani 1923	Monoecious	15	8	45	·036 papillate.	India: Sikkim Himalaya.
helmsii Stephani 1916	Monoecious	30	6	50	·045 dark, echinate.	Australia: New Zealand.
hispidus Stephani 1916	Monoecious	15	2	40	·036 black, hispid.	America: Martinique.
indicus Stephani 1916	Monoecious	15	3	20	·036 dark, asper.	India: Mysore.
kerneri Stephani 1916	Monoecious	20	5	50	·036 dark, hispid.	America: Brasilia, Sao Paulo.

Chart showing differences between those species of Anthoceros Linn. 1753 with cavernous thallus and dark spores—contd.

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
kahuensis Stephani 1916	Monoecious	15	6	60	·036 dark, hispid.	Asia : Hawai.
lamellatus Stephani 1916	Dioecious	5	4	20	·045 black, hispid.	America : Brasilia ; Rio Janeiro.
laminiferus Stephani 1893	Monoecious	20	5	30	·036 dark papillate.	New Zealand.
longicapsulus Stephani 1916	Monoecious	10	5	60	·045 black, hispid.	Britannia : Madeira.
longii Stephani 1916	Monoecious	5	2	20	·045 black, asper.	India : Himalaya : Simla.
macounii Howe 1898	Monoecious	4	1	60	·057 dark, dense muricate.	America.
macrosporus Stephani 1916	Monoecious	20	4	40	·054 dark, papillate.	India : Bhor Ghat ; Kodaikanal.
mandoni Stephani 1916	Monoecious	20×3	4	35	·045 black, hispid.	Africa : Insula Madeira.
meggitti Khanna 1037	Dioecious	12-17×7-8	6-7×·9	46-55×·5	·04 granular papillate.	Burma : Taunggyi.
multifidus Linn 1753	Monoecious	5	3	20	·036 brown, hispid.	Europa : Fennia, Silesia.
myriandroecius Stephani 1916	Dioecious	12	2	30	·054 dark long papillae.	Africa : Ruanda.
nagasakiensis Stephani 1916	Monoecious	5	3	25	·045 dark, hispid.	Japonia : Nagasaki.
niger Stephani 1916	Monoecious	15	3	35	·054 black, muricate.	Asia : Philippinae Insulae.
notothyloides Stephani 1923	Monoecious	10	3	15	·036 black, hispid.	India.
peruvianus Stephani 1916	Monoecious	10	4	40	·036 black, hispid.	America : Peruvia ; Lima.

Chart showing differences between those species of Anthoceros Linn. 1753 with cavernous thallus and dark spores—contd.

	Sexuality	Plant size	Involucre	Capsule	Spore	Distribution
pichinchensis Spruce 1885	..	..	3	30	·025 minute scabrata.	America : Andes ; in monte Pinchincha.
punctatus Linn. 1753	Monoecious	5	3	30	?black, hispid.	Europa.
ravenelii Austin 1875	Monoecious	10	2	10	?black, vermiculate.	America : Louisiana.
sambesianus Stephani 1916	Monoecious	10	3	35	·036 black, echinate.	Africa : Sambesi River, Boroma.
skottsbergii Stephani 1916	Monoecious	20×5	5	40	·45 dark, hispid.	America : Insula Chiloe.
spongiosus Stephani 1916	Monoecious	15	5	60	?dark, hispid.	Asia : Hawai.
stableri Stephani 1895	Monoecious	10	3	20	·042 dark, echinate.	Britannia : Westmore-land.
stephanii Khanna 1937	Monoecious	8	3	30	·036 dark, muricate.	Asia : Himalaya.
subtilis Stephani 1916	Monoecious	5	2	12	·036 dark, hispid.	Asia : India, Mangalore.
telaganus Stephani 1916	Dioecious	5	5	20	·045 dark, minute papillae.	Asia : Java.
tubinatus Stephani 1909	Monoecious	..	2	30	·045 dark, papillate.	America : Mexico, Rio Blanco.
venosus Lindenberg et Gottsche 1845.	Monoecious	15	4	25	·036 dark.	America : Mexico.
volkensii Stephani 1916	..	25	..	..	..	Africa : Kilimandscharo
wrightii Stephani 1916	Monoecious	15	3	50	·036 dark, lightly hispid	America : Cuba.

A. fausiformis Austin 1877, *A. gross-involucratus* Stephani 1923, *A. indicus* Stephani 1916, *A. laminiferus* Stephani 1893, *A. longii* Stephani 1916, *A. macounii* Howe 1898, *A. macrosporus* Stephani 1916, *A. myriandroecius* Stephani 1916, *A. nigra* Stephani 1916, *A. stephanii* Khanna 1937, *A. telaganus* Stephani 1916, and *A. tubinatus* Stephani 1909. The light brown colour of the spore separates the present form from *A. faurianus* Stephani 1916; *A. longii* Stephani 1916, and *A. nigra* Stephani 1916 ; the smaller size of the thallus together with its shape and dioecious nature from *A. indicus* Stephani 1916 and *laminiferus* Stephani 1893; the smaller size of the spore from *A. chevalieri* Stephani 1923, *A. macounii* Howe 1898, *A. mcrosporus* Stephani 1916 and *A. myriandroecius* Stephani 1916; the smaller size of the involucre and shape of the thallus from *A. gross-involucratus* Stephani 1923 and *A. fusiformis* Austin 1877; the longer involucre and dioecious nature from *A. fertilis* Stephani 1916 and *A. cristatus* Stephani 1916 ; the smaller size of the thallus together with dioecious nature from *A. augustus* Stephani 1916; the dioecious nature together with the shape of the thallus from *A. stephanii* Khanna 1937 and *A. tubinatus* Stephani 1909; the shorter invalucre and smaller size of the spore from *A. telaganus* Stephani 1916. It is therefore necessary to create a new species for which the name *A. Chungii* sp. nov. is proposed.

Description.*

    Planta dioica. Pannis fusco-viridis commixa. Frons 4-10 longa, 3-5 lata. In medio depressa, margine elata. Multis angustis lobulis dirisa, linearibus vel aliquantium cuneatis. Cellae superficiales 0.035-0.045×0.01-0.025. Mediae frontis sectio transversa 10-16 cellis excelsa. Magnae lacunae. Involucre 2-4.5 longa, 0.3-0.5 lata, aspectu ovalis, in apicem acuminata, ore lobulato. Capsule 15-45 longa, 0.2-0.3 lata, Stomata 0.05-0.07×0.02-0.03, cellis inequalibus custodita. Sporae 0.03-0.04 subfuscae, granulatae papillatae. Pseudoelatores fusci, 2-6 cellis muniti. Antheridia 2-4 in caverna conglobata.

References

CHOPRA, R.S.—(1938) Notes on Hepatics 1. South India. Proc. Ind. Acad. Vol. vii: pp. 239-251.

KASHYAP, S.R.—(1915) Notes on New and Little-known Western Himalayan Liverworts. No. 3. New Phytologist, Vol. xxv, pp. 1-18.

    ,,   —(1929) Liverworts of the Western Himalayas and the Punjab, Plain, Part I. The University of the Punjab Lahore.

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KHANNA, L.P.—(1937) On two new species of *Anthoceros* Linn. 1753 from Southern Shan States, Burma, with a Comparative chart of the Dioecious dark spored species of the Genus. Jour. Bom. Natur. Hist. Soc. Vol. xxxix, No. 2, pp. 358-360.

MITTEN, W.—(1860-1861) Hepaticae Indiae Orientalis. Jour. Proc. Linn. Soc. Vol. v, Nos. 18, 19, pp. 89-128.

STEPHANI, F.—(1912-1917) Species *Hepaticarum* pp. 972-1007

,,   —(1917-1924) Species *Hepaticarum* pp. 425-429.

ON A FORM OF *ANABAENOPSIS* FROM MADRAS *

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(With one plate and fifteen text-figures).

The alga forming the subject of this note was collected in April, 1934, from the estuarine region of the River Cooum at Madras. This estuary is connected with the sea only during portions of the year and the rest of the period it is separated from the sea by a sand bar. At the time of collection of the alga, the estuary was not connected with the open sea, and the water was stagnant and highly brackish. The alga was found occurring here as a floating plankton imparting to the water a greenish appearance. It was especially abundant in certain stagnant portions of the water, between some rocks near the bank, where it formed a pale green scum on the surface. The alga was collected with the help of a plankton net and was preserved on the spot in 5% formalin in the estuarine water.

On the following day, some of the alga preserved in formalin was washed in several changes of water and then gradually taken up through the grades of alcohols into 85% alcohol. The washing as well as the changes of alcohols were all done with the help of a centrifuge. Smear preparations of the alga were made from 85% alcohol as per method described for protozoological perparations by McClung (1929, p. 397 et seq). A slide is smeared with a thin layer of Mayer's albumen fixative and a drop of the 85% alcohol containing the alga is pipetted on to the surface of the slide. The alcohol while it evaporates distributes the organisms and coagulates the albumen. When the albumen is just beginning to dry up, the slide is dipped in a jar of 90% alcohol for further

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hardening of the albumen. From 90% alcohol the slide is taken down through the alcohol grades back into water and then stained with iron-alum haematoxylin. A few slides were stained in Delafield's haematoxylin, and a few in safranin, but the iron-alum haematoxylin preparations were found to be the most satisfactory.

The trichomes of the alga vary considerably in length and are always closely coiled into a spiral of $\frac{1}{2}$-16 turns (Pl. XIII, Fig. 1, Text-Figs. 1-9). The spirals are on the average about 30-80 μ in diameter. The direction of the coiling of the trichome is either clockwise or anti-clockwise, but the coiling of any one particular individual is in one direction only; there is no reversal in the direction of the coiling in the course of its spiral. However, a false impression of a reversal in the coiling is given when the trichomes are examined under a cover glass after the water underneath the cover is allowed to dry up somewhat (Text-figs. 5, 6, 8). The trichomes then, owing to the pressure of the cover-glass, become flattened out, and their spirals become elongated and are thrown into many folds, showing several changes in the direction of the coiling, thus giving a false impression of reversals of the spirals. (Text-figs. 5, 6, 8).

The trichomes of the alga are 7.6—11.4 μ thick (without the mucilage), and the thickness of each individual trichome is more or less uniform throughout its length. The cells comprising the trichomes are short and barrel-shaped and are slightly rounded between the cells (Text-figs. 10, 12—15). Their contents are blue green in colour and are slightly granular with a few pseudovacuoles. They are as a rule slightly broader than long but often they are as long as broad, and are distinctly longer just before division. The division of the cells takes place by the usual constrictive division about the middle of the parental cell (Text-figs. 11—15). The dimensions of the cells are 7.6—(9.5)—11.4 μ broad, and 5.7—(7.6)— 9.5 μ long.

The protoplast of each cell is surrounded by a wall composed of three investments, an inner, a middle and an outer layer. The inner layer forms a closely investing, thin, but firm layer round the protoplast, and is continuous with the transverse septum separating the cells of the trichome. When a filament is treated with iodine and sulphuric acid or with chlor-zinc iodide, this layer remains unaffected indicating that it contains no cellulose. Similarly when treated with ruthenium red, it remains unaffected showing that no pectin either is present. The middle layer is also thin, but is decidedly thicker than the inner one. This layer, unlike the inner one, is stained distinctly blue with iodine and sulphuric acid and violet with chlor-zinc iodide, indicating a clear cellulose nature. When treated with ruthenium red,
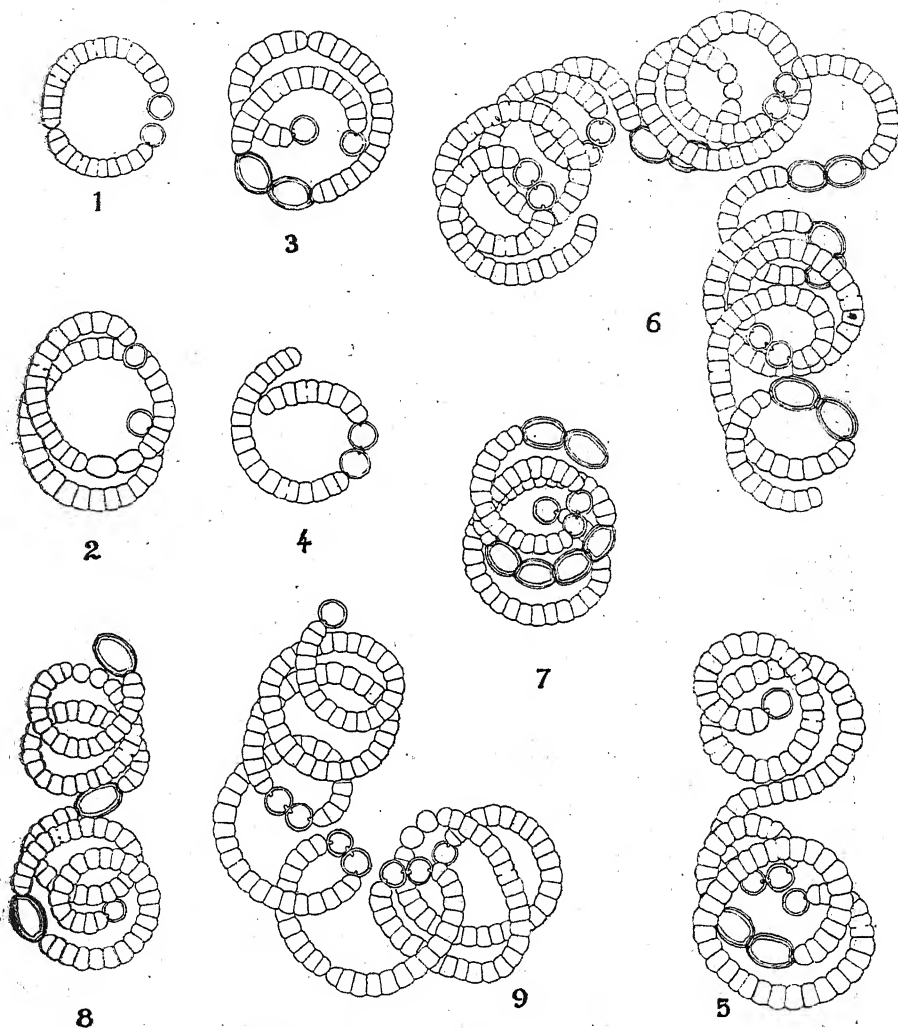
Text - figs. 1—9. Text-fig. 1. A short trichome with a heterocyst at each end and a developing pair of intercalary heterocysts. Text-fig. 2. Trichome with a heterocyst at each end and a developing pair of intercalary spores. Text-fig. 3. Similar to Text-fig. 2 but with spores fully mature. Text-fig. 4. A trichome without any heterocyst at either end. Text-figs. 5, 6 and 8. Trichomes showing several reversals in the coiling due to pressure of the alga under the cover-glass. Text-fig. 7. Trichome with a pair of spores at one end and a heterocyst at another and a row of four spores in the middle. Text-fig. 9. A trichome with a number of pairs of intercalary heterocysts in various stages of development. (All figures × 290).

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however, it remains unaffected indicating that no pectin is present. When stained with iodine, this layer is seen as a continuous one round the whole length of the trichome and is not interrupted opposite the transverse septum, as in *Anabaena* (Fritsch 1905, Spratt. 1911), where it forms a sort of cylindrical sheath about each cell and is not continuous with those of the neighbouring cells.

The outer layer is very broad and mucilaginous and forms a thick and highly transparent sheath round the trichome. This sheath is seen clearly when the alga is mounted in indian ink (Pl. XIII, Fig. 2), or is strained with Bismark brown, methylene blue or gentian violet. A very good idea of the exact nature of this sheath is obtained from permanent smear preparations made by the method already described. In these preparations, as a result of the dehydration of the alga when taken through the various grades of alcohol, the mucilaginous sheath becomes very much contracted and is then seen, not as a continuous thick layer, as one sees in indian ink, or with any of the stains, but as a series of wing-like expansions around each cell of the trichome, separated from one another by a more or less deep constriction opposite each transverse septum (Pl. XIII, Figs. 3-4, Text-fig. 11). Each of these individual wing-like portions represents the outer mucilaginous layer of the cell-wall highly contracted as a result of the dehydration. Under ordinary conditions, this mucilaginous layer of the cell-wall is full of water and is highly swollen and abuts closely on to the corresponding layers of the adjacent cells, with the result that the entire trichome appears to be surrounded by one continuous thick mucilaginous sheath (Pl. XIII, Fig. 2). It is only when dehydrated that the composite and discontinuous nature of the mucilaginous sheath becomes evident. In other words, what appears to be one continuous sheath round the whole length of the trichome is really a series of separate cylindrical mucilaginous portions placed end to end, each cylindrical portion being secreted by the respective cell which it envelopes. This secretion of mucilage by the individual cells, however, does not seem to be quite uniform all round, for there is always present opposite each transverse septum a deep construction indicating the absence of mucilage there (Pl. XIII, Figs. 3, 4, Text-fig. 11). The secretion of mucilage by each cell seems to be greatest opposite to its middle region and to gradually diminish towards the two ends and to be completely absent at the region of the transverse septum. Further, the mucilage secretion appears to be greater on the outer (convex) side of the trichome than on its inner (concave) side, since the contracted wing-like portions of the cells are larger and broader and more or less rectangular on the outer side and are smaller and narrower and more or less triangular on the inner side.

The above described composite nature of the mucilaginous sheath, owing to its secretion by each individual cell, is not a new

observation. Fritsch (1905) in his study of *Anabaena* has already observed a similar feature. He says (Fritsch, 1905. p. 201) "Treated with Vesuvin it (*the mucilaginous invest-ment*)* turns brown and is seen to consist of a number of successive layers. The inner most, and therefore most recent, of these closely follows the outline of the cell-sheaths of the individual protoplasts and thus presents a moniliform appearance, indicating the excretive activity of each cell". The present observations confirm fully the above observations of Fritsch regarding the excretive activity of each cell and afford further a clear demonstration regarding the composite nature of the mucilaginous sheath round the trichome.

Heterocysts

The heterocysts in this alga, are both terminal and intercalary. In the case of the former they are usually single, situated one at each extremity of the filament (Pl. XIII, fig. 3 Text-figs. 1—3, 5, 9.), while, in the latter case, they are usually in pairs (Text-figs. 4, 5, 6, 7, 9, 14). Each of the fully formed heterocysts is more or less spherical in shape and its contents are homogeneous and greyish green in colour. They are 9.5—(13.3)—17.1 μ in diameter and are provided with a thick wall composed of two envelopes, an inner and an outer. A fine refractive granule is usually visible at one end, or often even at both the ends, of the mature heterocyst (Pl. XIII, Fig. 3—4, Text-figs. 11, 14).

The heterocysts in this alga originate always in pairs in an intercalary position, through the division of any two adjacent, intercalary, vegetative cells. These two cells divide simultaneously, but unequally, each cell cutting off a smaller portion on the side on which it is in contact with the other (Text-fig. 12). The two small cells which are thus cut off, develop in contact with each other into the two heterocysts (Text-figs. 1, 3, 6, 9, 11-15). The details of their development are as follows. Their contents gradually lose their granular appearance and become homogeneous. The middle layer of their cell wall becomes thickened all round, except at the two ends where the cells are in contact with their neighbouring cells. At these two ends a small pore-like unthickened portion becomes evident, the contents appearing to be communicating with those of the neighbouring cells—the vegetative sister cell on one side and the second heterocyst on the other. Meanwhile, owing evidently to the transformation of the protoplast into a homogeneous substance and probably also to a change in the nature of the middle layer of the cell-wall, the secretion of the mucilage by the protoplast is stopped. Consequently, the mucilaginous sheath gets separated as a lose sheath round the heterocyst (Text-fig. 10) and is finally lost. Thus the heterocysts when they are fully mature, possess usually no mucilaginous sheath (Pl. XIII, Figs. 3-4, Text-fig. 11). When the walls become further

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*The words within the brackets are by the present author.
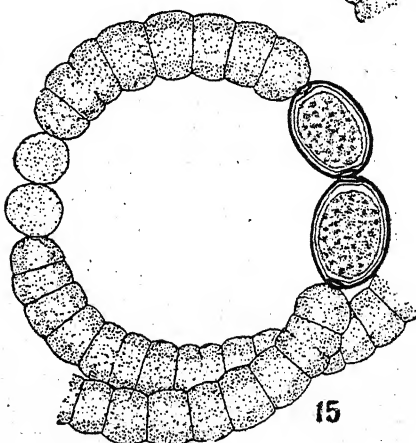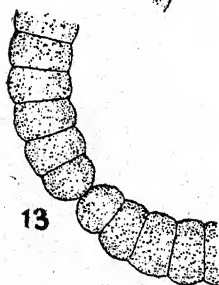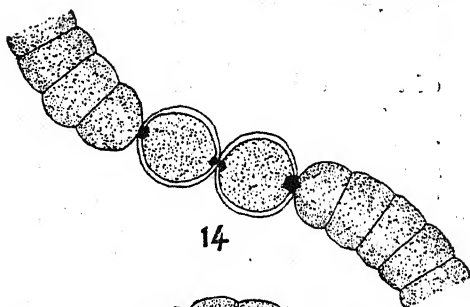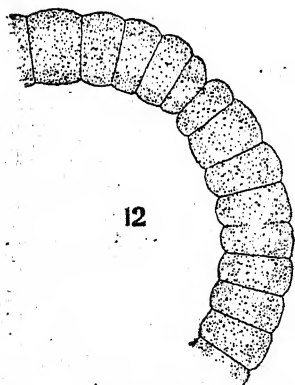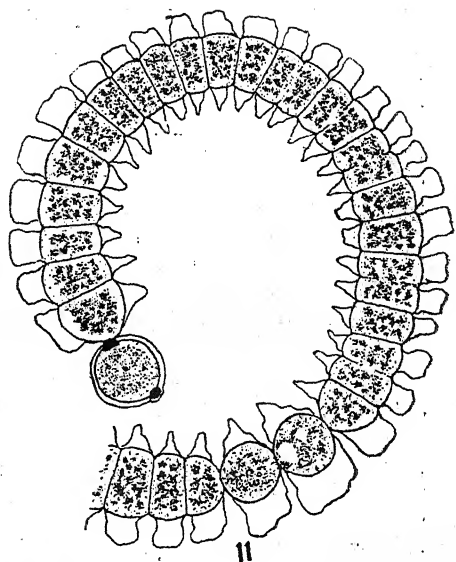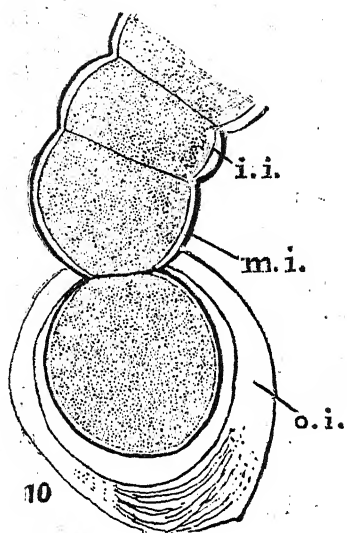
Text-Figs. 10—15. Text-Fig. 10. Portion of the trichome after treatment with iodine and sulphuric acid showing the inner and middle investments of the cell and the loose mucilaginous investment of the hetero-cyst. The outer mucilaginous investment of the cells of the trichome

thickened, a fine refractive granule makes its appearance opposite each of the pores and becomes prominent, as the heterocysts mature (Pl. XIII, Figs. 3-4, Text-figs. 11, 14).

Each of the newly organized intercalary heterocyst has two large granules one on either side, where it is in contact with the neighbouring cells (Text-figs. 4, 7, 9, 14). These two granules are generally equal in size, but often the granule on the side on which the heterocyst is in contact with the other heterocyst is somewhat smaller (Text-fig. 14). When these heterocysts become terminal owing to the fragmentation of the trichome between them, the granule now on the free end of the heterocyst, becomes gradually smaller and smaller and ultimately disappears. Thus it is often difficult to observe a granule at the free end of a terminal heterocyst, though the one at the other end is always prominently seen (Pl. XIII, Figs. 3-4).

Each of the fully formed heterocyst is more or less spherical in shape and has a thick wall composed of two envelopes, an inner and an outer. When treated with iodine and sulphuric acid, or with chlor-zinc iodide, the inner of the two envelopes shows a clear cellulose reaction, while the outer layer remains unaffected. When a trichome is stained with ruthenium red, by a prolonged immersion in it, only the inner layer is stained lightly, indicating the presence of pectin as well in this layer. The outer is layer unaffected even in this case.

The trichomes of this alga generally fragment into a number of smaller trichomes. The fragmentation takes place usually between a pair of intercalary heterocysts, with the result that the heterocysts become terminal in each of the daughter trichomes. Frequently, however, the trichomes fragment at other places, *e.g.*, between a vegetative cell and the pair of intercalary heterocysts or between any two vegetative cells. In such cases, the daughter trichomes do not possess a heterocyst either at one or even at both ends (Text-figs. 4, 6).

Though the heterocysts in this alga may be found both in a terminal and in an intercalary position, their origin is always intercalary. They never originate in a terminal position. Further the two intercalary heterocysts are not derived by the division of a single vegetative cell, but by an unequal division of two adjoining vegetative cells. In other words, the two heterocysts are not sister heterocysts.

The formation of heterocysts here described is quite similar in all respects to that described for various species of *Anabaenopsis* by previous authors. The stages in the formation of the heterocysts in the present alga agree very well with those figured by Miller (1923) in *A. Elenkini* (Miller 1923, p. 118, figs. 1,3,4), by Aptekarj (1926) in *Anabaenopsis Arnoldii* (Geitler, 1930-32, Fig. 519) and by Taylor (1932) in a number of species of *Anabaenopsis* (Taylor, 1932, Pl. 39, Figs. 1,4,5,6,8,9, 10,11,13 and 14).

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mucilaginous investments of the individual cells. Note the prominent granules at both the ends of the heterocyst and the absence of mucilage sheath round the heterocyst. Text-fig. 12-15 Portions of trichomes

Spores

The spores are usually intercalary in position and are formed mostly in pairs (Text-figs. 3, 6, 7, 15). They are, as a rule, remote from the heterocysts, and are separated by a few or more vegetative cells. When fully ripe they are ellipsoid in shape and are 13·3—(15·2)–19·0 μ broad and 17·1–(19·0)–22·8 μ long. They possess a thick wall, composed of two envelopes, an inner and an outer (Pl. XIII, Fig. 2, Text-figs. 3, 6-8, 15). Their contents, when mature are highly granular and greyish green in colour.

The development of the spores takes place as usual from any ordinary vegetative cell, and here also, as in the case of the heterocysts, the cells giving rise to them are always intercalary in position and never terminal. Any two intercalary vegetative cells lying adjacent to each other increase in size (Text-fig. 2) more or less simultaneously and their contents become more granular in appearance. Their walls become gradually thickened, and their ends get rounded off and eventually grow into mature spores (Text-figs. 2, 3, 6, 7, 15).

The fully developed spore has two envelopes, an inner, thin and transparent, and an outer, very thick, smooth and faintly striated (Pl. XIII, Fig. 2, Text-fig. 15). These two envelopes are derived from the two respective envelopes of the vegetative cells and show more or less the same reactions to iodine and sulphuric acid or chlor-zinc iodide. The outer wall of the spore, however, gives a much clearer reaction for cellulose than that given by the corresponding layer in the vegetative cell. It also gives a faint reaction for pectin when stained with ruthenium red. Evidently this layer is made up of cellulose with a small quantity of pectic material.

Though the spores are generally formed in pairs and in an intercalary position (Text-figs. 2, 3, 5, 6, 7, 15), deviations from this rule are frequently seen. Occasionally only a single spore is formed evidently due to the failure of one of the two adjoining cells to develop into a spore (Text-fig. 8). So also occasionally, due to the simultaneous development of two pairs of adjoining cells, a row of four spores is formed (Text-fig. 7). The fragmentation of the trichome may take place between a pair of originally intercalary spores and transform them into terminal spores, one at each end of the trichome (Text-fig. 8). Occasionally again, two spores may be found at one or both ends of the trichome due to the fragmentation of the trichome between a vegetative cell and a pair of intercalary spores (Pl. XIII, Figs. 2, 4, Text-fig. 7).

Multiplication

Multiplication in this alga takes place by the fragmentation of the trichome into two or more smaller fragments. The fragmentation is effected usually between a pair of intercalary

heterocysts, their point of attachment, probably affording the easiest portion for breaking apart. However, as already pointed out, fragmentation may take place in other positions also—(i) between a vegetative cell and a pair of heterocysts, (ii) between a pair of spores, (iii) between a vegetative cell and a pair of spores or (iv) merely between two vegetative cells.

Systematic position of the alga

From a description of the alga given above, it is quite clear that the alga under consideration undoubtedly belongs to the genus *Anabaenopsis*, as shown by the usual terminal position of the heterocyst, by the paired intercalary development of the heterocysts and spores and by the development of spores usually away from the heterocysts. In the shape of its cells and heterocysts, it agrees very well with *Anabaenopsis Arnoldii* Aptek. But, the dimensions of the present alga are larger than those of the other forms in all respects. In the number of spirals ($\frac{1}{2}$—16) it agrees somewhat with the Russian form ($\frac{1}{2}$—9) but exceeds it in the greater number, as well as in the greater diameter of the coils (30—80μ). Further, in the average thickness of its trichomes, it is much thicker than the Russian as well as all the other forms, the nearest approach to it being the Philippine form of Taylor (1932). The thickness of the trichome in the present case is 7.6—11.4 μ whereas those of the Russian and the Philippine forms are only 6.5—9.0 μ and 7.5—9.4 μ respectively. However, the more marked differences from any of the described forms are the dimensions of the heterocysts and the spores, which exceed those of any of the forms described till now. On an average, the heterocysts of the present alga are 13.3 μ in diameter, while those of the Philippine, the Russian and the African forms are on an average only 7.9μ, 9.2μ and 9.8μ respectively. The dimensions of the spores of the present alga are 13.3 −(15.2)— 19.0 μ broad and 17.1 —(19.0) — 22.8μ long, while the largest size for any of the forms described till now is only 12 — 14 μ broad and 16 — 18 μ long [*i.e.*, in the Javanese form of *Anabaenopsis Arnoldii* Aptek. (=*Anabaenopsis circularis* var. *javanica* (Woloszy.) Elenk.)] Thus from the foregoing, it is quite clear that the alga under consideration is undoubtedly much larger than any of the forms of *Anabaenopsis Arnoldii* Aptek, so far described and may therefore, be regarded as a new variety of *A. Arnoldii* which may be called *Anabaenopsis Arnoldii* Aptek. var. *indica* var. nov.

Description of the alga

Anabaenopsis Arnoldii Aptek. var. *indica* var. nov.

Trichomes elongated, spirally coiled; coils $\frac{1}{2}$—16 in number; diameter of each coil 30—80μ; trichome 7.6—11.4μ; in diameter; cells short and barrel shaped and slightly rounded between the cells; cells usually broader than long, but distinctly longer just before division; cells 7.6—(9.5)—11.4μ broad and 5.7—(7.6)—

9·5μ long; cell-contents blue green in colour and slightly granular, with a few pseudovacuoles; heterocysts terminal or intercalary; origin of heterocyst always intercalary in pairs, by the development of two cells derived by an unequal division of two adjoining vegetative cells; heterocysts spherical, 9·5—(13·3)—17·1μ in diameter; contents pale blue green in colour; wall fairly thick, with a distinct basal granule at one or even both ends; spores intercalary or terminal, usually away from the heterocysts; origin of the spores always intercalary and never terminal; spores ellipsoid in shape and are 13·3—(15·2)—19·0μ broad and 17·1—(19·0)—22·8μ long; spore wall very thick and faintly striated; contents highly granular and greyish green in colour; multiplication by fragmentation of the trichome, usually between a pair of intercalary heterocysts.

Habitat :—Found as a water-bloom in the estuarine region of the River Cooum at Madras, India.

General Considerations

The genus *Anabaenopsis* was established by Miller (1923) in 1923 raising Woloszynska's (1913) section *Anabaenopsis* of the genus *Anabaena* to a generic status. He gives the following diagnosis for the new genus :—" Trichomata brevia, spiraliter contorta, Anabaenae similia, sed utroque fine heterocysta terminata. Heterocytae novae semper binae e cellulis contiguis nascuntur et inter eas trichomata in partes fere aequales dissolvunter. Sporae (ubi notae) sphaericae, sive ellypticae a heterocystis remotae." (Miller 1923, p. 125.) He gives also a brief German summary wherein he states :—" Beschreibung einer neuen Cyanophycee (Centralrussland), die zur einen neuen Gattung *Anabaenopsis* gehört und sich von der Gattung *Anabaena* durch die stets terminalen, an beiden Enden der kurzen, spiralig gebogenen Fäden sich befindenden Heterocysten unterscheiden. Die Heterocysten werden immer paarweise durch ungleiche Teilung zweier nebeneinander liegender Zellen gebildet und zwischen ihnen zerfallen die Fäden in zwei ungefähr gleiche Teile." (Miller 1923, p. 126.) Thus Miller describes two important features as characteristic of his new genus *Anabaenopsis*, (1) the presence of a heterocyst at each end of the trichome and (2) the development of paired intercalary heterocysts always by an unequal division of two adjoining vegetative cells.

Aptekarj (1926) described in 1926, a new species of *Anabaenopsis* by name *Anabaenopsis Arnoldii* Aptek. and figured the characteristic method of formation of the paired intercalary heterocysts, through the unequal division of two adjoining vegetative cells (Ref. Geitler (1930-32), Fig. 519), similar to that originally described by Miller (1923) in *A. Elenkini* Miller.

Geitler (1930-32), while describing the genus *Anabaenopsis* expresses the view that the differences between *Anabaenopsis* and *Anabaena* are not substantial and doubts whether a separate genus could be maintained. He, however, treats *Anabaenopsis* as a separate genus and states in his general diagnosis of the genus that the heterocysts in this genus are as a rule one at each end of the trichome and that no intercalary heterocysts are present except in *Anabaenopsis Arnoldii* Aptek. He gives this latter species, a doubtful position and places it in an appendix of the genus *Anabaenopsis*, expressing the opinion that *A. Arnoldii* probably belongs to *Anabaena*, on account of the frequent absence of the terminal heterocysts in this alga. But, he points out at the same time that this species should be considered as an *Anabaenopsis* because of the characteristic method of origin of the heterocysts, even though the species lacked frequently the terminal heterocysts. Thus Geitler (1930-32) seems to be of the opinion that the peculiar origin of the paired intercalary heterocysts is evidently a more distinguishing feature of the genus, than the presence of a heterocyst at each end of the trichome. He states: "Die Art liesse sich aus praktischen Grunden mit mehr Recht zu *Anabaena* stellen, da das Merkmal der ausschliesslich endstandigen Heterocysten fehlt. Doch zeigt die charakteristische Entstehungsweise der Heterocysten (siehe die Figur), dass sich die Art zwanglos als eine *Anabaenopsis* vom Typus *A. circularis* auffassen lasst, bei welcher das Auseinanderbrechen der Trichome zwischen den Schwesterheterocysten verzogert ist. Soweit bekannt, kommen solche Schwesterheterocysten bei *Anabaena*, zumindest nicht regelmassig, nicht vor." (Geitler 1930-32, p. 810-811).

Taylor (1932) in describing a number of species of *Anabaenopsis* found that in most of his specimens the heterocysts were formed by the paired development of two small cells, each derived as a result of an unequal division of two adjoining vegetative cells. He points out, "Aptekarj (1926) stresses the paired intercalary heterocysts seen in his plant (*Anabaenopsis Arnoldii* Aptek.*) as a feature to distinguish it from the other *Anabaenopsis* species; this is not a character that can be thus applied. It appears that, in *Anabaenopsis,* the heterocysts are formed in pairs by the unequal subdivision of two vegetative cells, each cutting off a small portion on the adjoining ends. These two portions then enlarge and mature in contact into the characteristic heterocysts of the species." (Taylor 1932, p. 457-458.) Thus this author also seems to be of the opinion that the paired intercalary development of the heterocysts always by an unequal division of two adjoining cells is a feature very characteristic of the genus *Anabaenopsis*.

From the foregoing it may be seen that all the four authors who have dealt with the genus, viz., Miller (1923), Aptekarj

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*The words in the brackets are inserted by the author.

(1926), Geitler (1930-32) and Taylor (1932), have expressed in
a way that the peculiar method of origin of the paired intercalary
heterocysts by an unequal division of two adjoining vegetative
cells is the more characteristic feature of the genus than the
mere terminal position of the heterocysts.

Bharadwaja (1933), while describing a species of *Cylindro-
spermum* with heterocysts at both ends of the trichome expresses
the opinion that *Anabaenopsis* can scarcely stand as a genus
separate from *Anabaena* simply because of the presence of a
heterocyst at each end of the trichome, since such a feature is seen
in certain species of *Anabaena* also (*Anabaena echinospora* Skuja).
He expresses the opinion that, if the presence of a heterocyst at
both ends of the trichome should form a sufficient basis for the
separation of *Anabaenopsis* from *Anabaena*, the same procedure
will have to be adopted in the case of those species of *Cylindros-
permum* which possess a heterocyst at both ends. This objection
would, no doubt, be quite valid, if Miller (1923) had based his
new genus solely on the single feature of the occurrence of a
heterocyst at each end of the trichome. But, as pointed out
already, Miller (1923) bases his genus not only on the pre-
sence of a heterocyst at both ends of the trichome, but also
on the method of origin of the two intercalary heterocysts,
through the unequal division of two adjoining vegetative cells.
Thus it is not the terminal heterocysts alone that distinguishes
this genus, but also the other and more important and
characteristic feature, viz., the method of origin of the paired
intercalary heterocysts.

Bharadwaja (1933) describes the formation of paired inter-
calary heterocysts in *Cylindrospermum muscicola* Kutz. var.
kashmirensis Bharadwaja. The method of origin of these paired
intercalary heterocysts is, however, quite different from that
described above for *Anabaenopsis*. In this *Cylindrospermum*,
the paired heterocysts originate by the division of a single
vegetative cell, while in *Anabaenopsis* the two heterocysts are
formed as a result of an unequal division of two adjoining cells.
In other words, the paired intercalary heterocysts in the
Cylindrospermum are sister-heterocysts, while in *Anabaenopsis*,
the paired heterocysts, are never sister-heterocysts, since they
are always derived from two different though adjoining vegeta-
tive cells.

Thus, in view of all the facts stated above, it looks as though
the best thing to be done under the circumstances is not to stress
on the presence of a heterocyst at each end of the trichome at all,
but to consider as the main distinguishing feature of the genus
Anabaenopsis, the characteristic method of origin of the hetero-
cysts, always in pairs, in an intercalary position, by the unequal
division of two adjoining vegetative cells. If this suggestion
should be accepted, only those species which show this peculiar

origin of the heterocysts should be included in the genus *Anabae-nopsis*, irrespective of the fact whether there are always terminal heterocysts or not.

According to this suggestion, the following species, where the method of origin of the paired intercalary heterocysts is already known, and conforms to the above definition can be straight away included in the genus *Anabaenopsis*:—

(1) *A. Elenkini* V. Miller (Miller, 1923).

(2) *A. circularis* (G. S. West) Wolosz. et Miller (Miller 1923).

(3) *A. Cunningtonii* Taylor (Taylor 1932).

(4) *A. Arnoldii* Aptekarj (Aptekarj 1926).

(5) *A. Arnoldii* var. *javanica* Taylor (*A. circularis* var. *javanica* (Wolosz). Elenk.) (Taylor 1932).

(6) *A. Arnoldii* forma *africana* Taylor (Taylor 1932).

(7) *A. Arnoldii* var. *indica*. var. nov.

The following species, however, can be included in this genus provisionally as forms not fully known until more information is available regarding the method of origin of the paired intercalary heterocysts in them:—

(1) *A. Nadsonii* Woronichin (Woronichin 1929).

(2) *A. Milleri* Woronichin (Woronichin 1929).

(3) *A. Tanganyikae* (G. S. West) Wolosz. et Miller (Miller 1923).

(4) *A. philippinensis* Taylor (Taylor 1932).

(5) *A. luzonensis* Taylor (Taylor 1932).

As regards *A. Raciborskii* Wolosz., according to Geitler (1930-32), the heterocysts in this species never originate in an intercalary position, but are formed out of the pointed end cell. He states (Geitler 1930-32, p. 809.) "Die Heterocysten entstehen niemals interkalar, sondern werden aus der zugespitzten Endzelle gebildet." Since the method of origin of the heterocysts in this species is quite different from that characteristic of the genus in being terminal and not intercalary, this species will have to be removed from the genus *Anabaenopsis*.

Finally, it must be stated, however, that very little is known regarding the method of origin of the heterocysts in *Anabaena*. It is just possible that the method of origin of the heterocysts in *Anabaena* may prove to be quite similar to that of *Anabaenopsis*, in which case the genus *Anabaenopsis* will have to be discarded and included as previously in *Anabaena* as a separate section *Anabaenopsis*. But until this is proved to be so, it is desirable to retain it as a genus quite separate from *Anabaena*.

Summary

A new variety of *Anabaenopsis Arnoldii* Aptek. is recorded from the estuarine region of the River Cooum at Madras.

A detailed account of the structure and nature of the different layers of the cell-wall, especially that of the outer-most mucilaginous envelope is given.

The origin and development of the heterocysts and the spores are described in detail.

The variations in the appearance of the trichome due to the irregularities in its fragmentation are explained.

It is pointed out, that though the presence of a heterocyst at each end of the trichome has been accepted as the chief feature of the genus *Anabaenopsis*, the other and more important feature mentioned by Miller (1923) in his original diagnosis of the genus, viz., the method of origin of the paired intercalary heterocysts by an unequal division of two adjoining cells, has not been given proper emphasis by the later authors. It is suggested that this characteristic method of origin of the heterocysts should be made the main distinguishing feature of the genus, the presence of a heterocyst at each end of the trichome being considered as a feature of only secondary importance.

Literature Cited

APTEKARJ, E. M. (1926).—De nova Cyanophycearum specie *Anabaenopsis Arnoldii* mihi. Bot. Mat., Inst. Sporov. Rast. Glav. Bot. Sad U. S. S. R. IV. (4): 41-55. Cited after Taylor (1932) and Geitler (1930-32).

BHARADWAJA, YAJNAVALKYA. (1933).—Contributions to our knowledge of the Myxophyceae of India. Annals of Botany. Vol. XLVII, 117-143.

FRITSCH, F. E. (1905).—Structure of the investment and spore-development in some Cyanophyceae. Beih. Bot. Centralbl., XVIII. Abt. 1., 194-214.

GEITLER, L. (1930-32).—Cyanophyceae, in Rabenhorst's Krypto-gamen-Flora von Deutschlands, Osterreichs und der Schweiz. XIV.

McClung, C. E. (1929). Hand-book of Microscopical Technique, New York.

Miller, V. (1923).—Zur Systematik der Gattung *Anabaena* Bory. (K Sistematike roda *Anabaena* Bory). Arch. Russ. Protistol. II, 116-126.

Spratt, E. R. (1911).—Some observations on the Life-history of *Anabaena Cycadeae*. Annals of Botany. XXV, 369.

Taylor, W. R. (1932).—Notes on the genus *Anabaenopsis*. Amer. Jour. Bot. 19, 454-463.

Woloszynska, J. (1913).—Das Phytoplankton einiger javanischer Seen mit Berucksichtigung des Sawa-Planktons. Bull. Internat. Acad. Sci. Cracovie., Cl. Sci., Math-et. Nat. B., Sci. Nat. 649-709. (Cited after Geitler (1930-32) and Taylor (1932).

Woronichin, N. N. (1929).—Materialien zur studien der Algen-vegetation in den seen der Kalundin Steppe. Bull. du Jard. Bot. Principal de l'u. R. S. S. Tome XXVIII, Nos. 1-2, p. 40.

Explanation of Plate XIII

Fig. 1. Photomicrograph showing a large number of trichomes of different lengths (X 47).

Fig. 2. Photomicrograph of a specimen mounted in indian ink, to show the outermost mucilaginous sheath (X 470).

Figs. 3. and 4. Photomicrographs of the smear preparation of the alga, stained in iron-alum haematoxylin, showing the wing-like mucilaginous investment around each cell. Note the presence of the prominent granule at the two ends of the terminal heterocysts in Fig. 5. (X 430).

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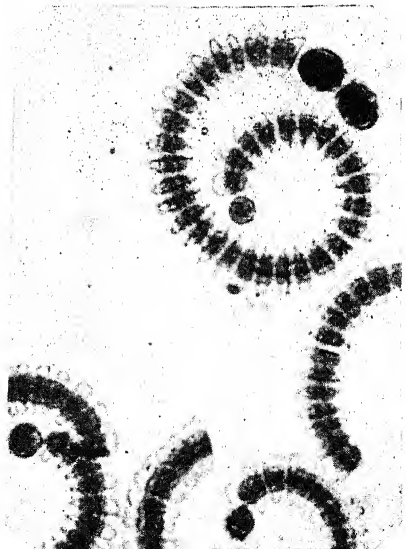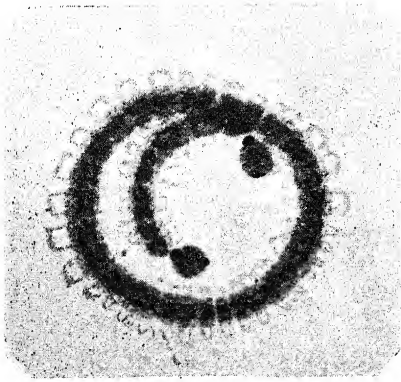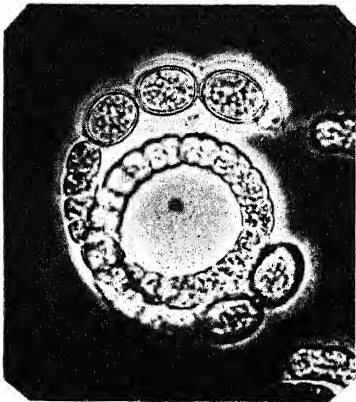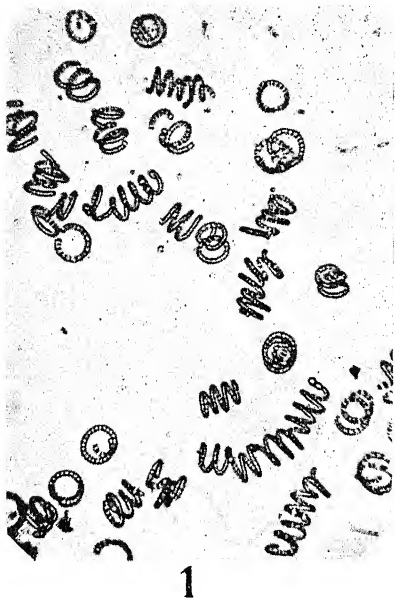
THE ZYGNEMOIDEAE OF THE CENTRAL PROVINCES, INDIA—I. *

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Communicated by Y. Bhâradwâja

During the earlier half of October, 1936, the writer made a fairly rich collection of algae from the Central Provinces, mainly from Katni, Durie, Jubbulpore, Itarsi and Hoshangabad. The investigation of these algae is being done in parts and they would be recorded in a series of papers.

No member of the Conjugales has yet been described from these Provinces and the present account relates to some of the Zygnemoideae. In all twenty-nine forms have been recorded and out of these six varieties and thirteen forms are new.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED.

(a) Zygnemaceae.

Genus *Zygnema* Agardh.

1. ***Zygnema globosum* Czurda** Czurda, in Pascher's Süsswasser-flora, Mitteleuropas, Heft 9, 1932, p. 109, Fig. 110.

Forma (Fig. 1, *A-D*)

Lat. cell., 27-30 μ; long. cell., 33-40 μ; crass. zygosp. spheric., 30-42 μ; dimen. oval zygosp., 36×42 μ; 32×43 μ; scrobiculations 3-4 μ broad and 1-2 μ apart.

Habitat:—In a stagnant pond (Adhar Tal) along with a sterile species of *Oedogonium*, *Aulosira Fritschii* and others, Jubbulpore.

This form is characterised by having smaller zygospores,

Genus *Spirogyra* Link.

2. ***Spirogyra varians* (Kütz.) Czurda** Czurda, *op. cit.*, 1932, p. 173, Fig. 177.

Forma

Lat. cell., 33-36 μ; long. cell., 40-82 μ; crass. zygosp., 30-36 μ; long. zygosp., 40-60 μ.

Habitat:—In a puddle on the rock quarry, Hoshangabad, along with *Spirogyra subsalsa* var. *macrospora*, sterile filaments of *Mougeotia*, *Spirogyra* sp., *Anabaena* sp., *Scenedesmus* sp., *Oedogonium* sp., *Pediastrum* sp. and *Gomphonema* sp.

This form has slightly broader cells.

3. ***Spirogyra decimina* (Mull.) Czurda** Czurda, *op. cit.*, 1932, p. 176, Fig. 181.

Forma (Fig. 1, *E*).

Lat. cell., (28-) 30-33 μ; long. cell., 85-100 μ; crass. zygosp., 35-36 μ; long. zygosp., 60-73 μ.

Habitat:—In a road-side puddle, along with *Spirogyra scrobiculata* var. *inflata*, sterile filaments of *Spirogyra*, *Zygnema* and a species of *Nitzschia*, Hoshangabad.

The form agrees with the type in all respects except that the zygospores are frequently longer, and the conjugation canals are shorter.

4. ***Spirogyra longata* (Vauch.) Czurda** Czurda, *op. cit.*, 1932, p. 178, Fig. 184.

Forma (Fig. 1, *F* & *G*).

Vegetative cells 2-6 times as long as broad, pale violet or hyaline; end-walls plane; chloroplast single. Conjugation lateral and very rarely scalariform; fructifying cells unswollen. Zygospores ellipsoidal, usually with rounded ends; exospore thin, smooth and colourless; mesospore thick, smooth and yellowish-brown; endospore indistinct.

Lat. cell., 36-42 μ; long. cell., 50-240 μ; crass. zygosp., 33-38 μ; long. zygosp., 40-63 μ, average 50 μ.

Habitat:—In a pool on hills, along with *Mougeotia recurva*, *M. tenuis* and others, Jubbulpore.

The form differs from the type in the broader filaments and zygospores, the latter with usually rounded ends. The scalariform conjugation is, however, very rare in this form.

5. ***Spirogyra scrobiculata*** (Stockmeyer) C z u r d a
Czurda, *op. cit.*, 1932, p. 182, Fig. 189.

Var. *inflata* var. nov. (Fig. 1, *H & I.*)
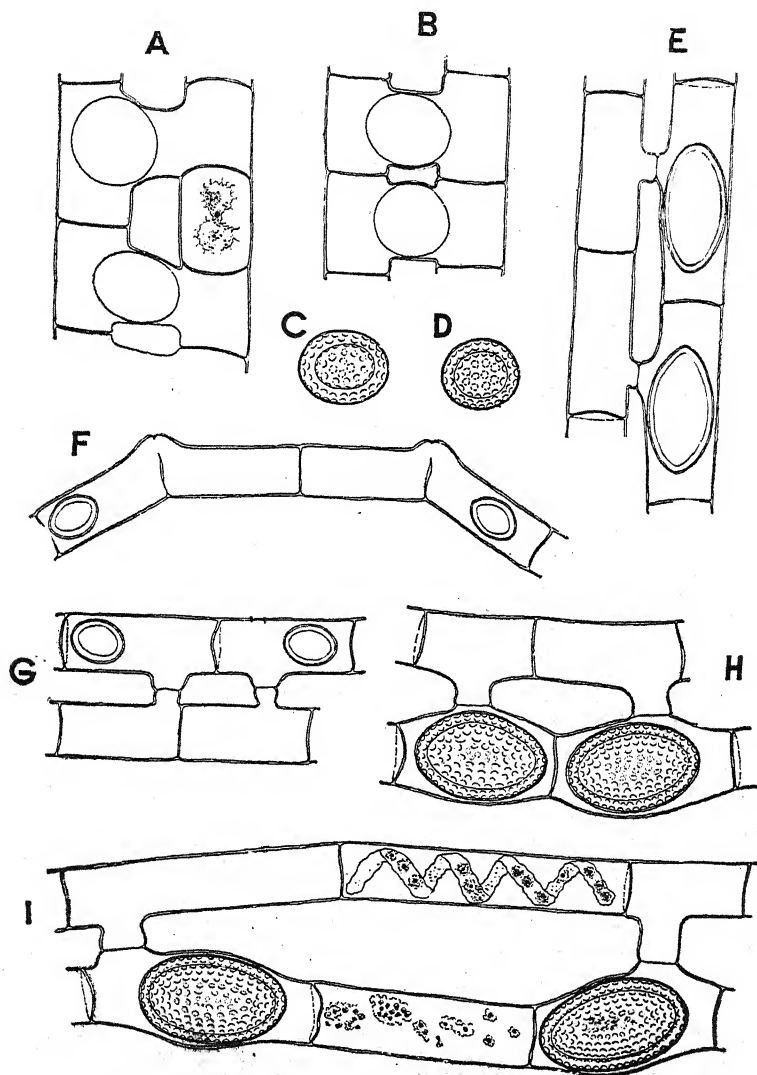
Fig. 1. *A & B*—Conjugating filaments with zygospores, *C & D*—zygospores showing the surface sculpture of *Zygnema globosum* Czurda forma; *E*—Conjugating filaments of *Spirogyra decimina* (Mull.) Czurda forma; *F & G*—the same of *Spirogyra longata* (Vauch.) Czurda forma; *H & I*—the same of *Spirogyra scrobiculata* (Stockmeyer) Czurda var. *inflata* var. nov. *A—E* × 278 ; *F & G* × 133 ; *H & I* × 237.

Vegetative cells cylindrical, 5-10 times as long as broad ; end-walls plane. Chloroplast single. Conjugation lateral ; conjugation canal formed mainly by male gametangia ; fructifying cells swollen alround. Zygospores ellipsoidal with rounded ends ; exospore thin, smooth and colourless ; mesospore thick, yellowish-brown with close-set scrobiculations.

Lat. cell., 30-33 μ ; long. cell., 140-230 μ ; crass. zygosp., 43-66 μ ; long. zygosp., 68-85 (-92) μ ; crass. cell. fructif., 42-58 μ.

Habitat :—In a rain-water pool near a river, Katni.

The variety agrees with the type in the plane end-walls, single chloroplast, scalariform conjugation, ellipsoidal zygospores with a thick, yellowish-brown and scrobiculated mesospore, but differs from the same in the fructifying cells being swollen alround, conjugation canal being formed mainly by the male gametangia, and in the bigger zygospores.

Another form exactly resembling the above variety, except having broader cells on the average, was collected in Hoshangabad from a rain-water puddle, along with *Spirogyra decimina* forma, sterile *Spirogyra*, *Zygnema* and *Nitzschia* sp. It has the following measurements :

Lat. cell., 30-40, average 33-36 μ ; long. cell., 115-155 μ ; crass. zygosp., 40-56 μ, average 52 μ ; long. zygosp., 60-82 (-85) μ ; crass. cell. fructif., 45-66 μ.

6. ***Spirogyra daedalea* Lagerheim** Czurda, *op. cit.*, 1932, p. 179, Fig. 186.

Var. *Jubbulporense* var. nov. (Fig. 2, *A—C*).

Vegetative cells upto three times as long as broad ; end-walls plane ; chloroplast single. Conjugation scalariform ; fructifying cells unswollen ; zygospores ellipsoidal ; exospore thin, smooth and hyaline ; mesospore thick, brown and closely reticulate-sculptured with a rib-line ; endospore indistinct.

Lat. cell., 37-41, average 40 μ ; long. cell., 66-237 μ ; crass. zygosp., 40-43 μ ; long. zygosp., 47-105, average 60-69 μ.

Habitat :—In a pool (Suba Tal) on hills, along with sterile samples of *Zygnema* and *Anabaena*, Jubbulpore.

The variety agrees with the type in the plane end-walls, single chloroplast, scalariform conjugation and ellipsoidal zygospores with a thick and brown mesospore, possessing a rib-line. But, it differs from the same in having slightly broader filaments, the fructifying cells being always unswollen and in the presence

of shorter zygospores, the latter possessing a mesospore that is closely reticulate-sculptured.

7. *Spirogyra subsalsa* K"tzing Czurda, *op. cit.*, 1932, p. 168, Fig. 171.

Var. *macrospora* var. nov. (Fig. 2, *D* & *E*).

Vegetative cells cylindrical, 5-9 times as long as broad; end-walls plane; chloroplasts 2-3. Conjugation scalariform; fructi-fying cells swollen alround. Zygospores ellipsoidal with pointed or sometimes with rounded ends; exospore thin, smooth and colourless; mesospore thick, smooth and yellowish-brown with a rib-line; endospore indistinct.

Lat. cell., 22-23 μ; long. cell., 66-210 μ; crass. zygosp., 28-33 μ; long. zygosp., 52-73, average 56 μ; crass. cell. fructif., 29-38 μ.

Habitat:— In a puddle on the rock-quarry of Hoshangabad, along with *Spirogyra varians* forma, sterile filaments of *Mougeotia, Spirogyra, Anabaena,* and species of *Oedogonium, Scenedesmus, Gomphonema, Pediastrum* and others.

The variety agrees with the type in the number of chloroplasts, scalariform conjugation, swollen fructifying cells, ellipsoidal zygospores with a thin smooth and hyaline exospore and a thick smooth and yellowish-brown mesospore with a rib-line, but differs from the same in possessing narrower cells, conjugation canals formed by both the gametangia and bigger zygospores with commonly pointed ends.

8. *Spirogyra dubia* K tz. Czurda, *op. cit.*, 1932, p. 188.

Forma (Fig. 2, *F.* & *G.*).

Vegetative cells upto three times as long as broad; end-walls plane; chloroplasts 2-3. Conjugation scalariform; fructifying cells swollen. Zygospores ellipsoidal to spherical; exospore thin, smooth and hyaline; mesospore thin, smooth and brown.

Lat. cell., 49-51 μ; long. cell., 60-115 μ; crass. zygosp., 46-50μ; long. zygosp., 62-66 μ; crass. cell. fructif., 56-66 μ.

Habitat :—In a stagnant puddle on the Hoshangabad rock-quarry, along with *Spirogyra submaxima* var. *lamellata, S. seti-formis* forma, *Sirogonium ventersicum* forma, *Aulosira Fritschii, Anabaena* sp., *Oedogonium* sp., and several others,

The form agrees with the type in all respects except that the zygospores are shorter.

Var. *polymorphis* var. nov. (Fig. 2. *H & I*)

Vegetative cells short, slightly longer than broad; end-walls plane; chloroplasts 2-3. Conjugation scalariform; fructifying cells swollen commonly on one or occasionally on both sides or sometimes not swollen at all. Zygospores ellipsoidal to spherical; exospore thin, smooth and hyaline; mesospore thin, smooth and yellowish-brown.

Lat. cell., 50-56 μ; long. cell., 60-75 μ; crass. zygosp., 50-63 (-66) μ; long. zygosp., 63-82 μ; crass. cell. fructif., 64-76μ.

Habitat:—In a pool (Madhav Tal) on hills, along with *Spirogyra pseudoneglecta* forma and a sterile species of *Spirogyra*, Jubbulpore.

The variety agrees with the type in the plane end-walls, the chloroplasts being 2-3, scalariform conjugation, swollen fructifying cells, ellipsoidal zygospores with a smooth brown mesospore, but differs from the same in the fructifying cells being commonly swollen on one and only occasionally on both sides or sometimes not swollen at all. Further it differs in having broader cells and on the average broader but shorter zygospores which are also spherical with a thin mesospore.

9. ***Spirogyra ternata* Ripart** forma **Rao** Rao, The Zygnemoideae of the United Provinces, India-I, *Journal of the Indian Botanical Society*, Vol. XVI, No. 5, 1937, p. 279, Fig. 4, *A*.

Lat. cell., 60-62 μ; long. cell., 48-82 μ; crass. zygosp., 50-66 (-73) μ; long. zygosp., 66-85 μ; crass. cell. fructif., 70-85 μ.

Habitat :—In Budagar lake, along with *Spirogyra neglecta* forma, Durie.

10. ***Spirogyra adnata* (Vauch.) Kütz.** forma **Fritsch** and **Rich**. Fritch and Rich, Contributions to our knowledge of the Fresh-water Algae of Africa, 7, Fresh-water algae (exclusive of Diatoms) from Griqualand West, *Transactions of the Royal Society of South Africa*, 1930, Vol. XVIII, Parts 1 & 2, p. 48, Fig. 12, *a-c*.

Lat. cell., 48-52 μ ; long cell., 33-100 μ ; crass. zygosp., 46-60 μ ; long. zygosp., 66-87 μ ; crass. cell. fructif., 60-65 μ.

Habitat :—In a pond, along with *Spirogyra columbiana* forma.and *Spirogyra neglecta*, Katni.
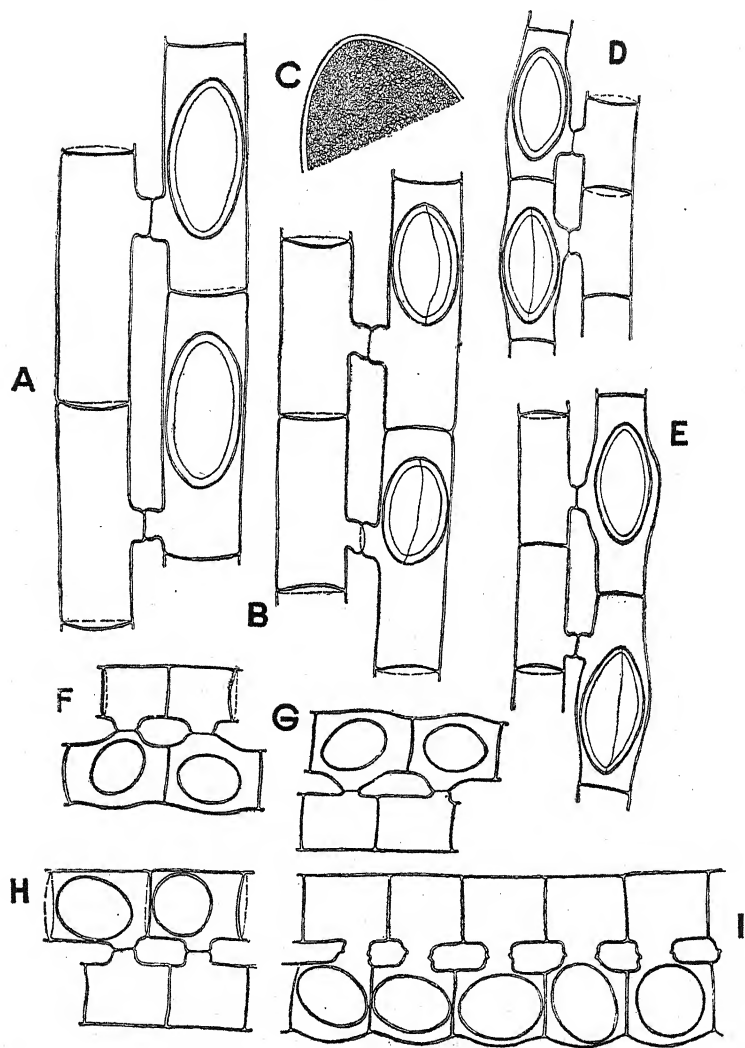
Fig. 2. *A & B*—Conjugating filaments with zygospores, *C* — a highly magnified zygospore showing the surface sculpture of *Spirogyra daedalea* Lagerheim var. *Jubbulporense* var nov. ; *D & E*—Conjugating filaments of *Spirogyra subsalsa* Kuetzing var. *macrospora* var. nov. ; *F & G*—the same of *Spirogyra dubia* Kütz. forma ; *H & I*—the same of *Spirogyra dubia* Kütz. var. *polymorphis* var. nov. *A & B* × 237 ; *C* × 587 ; *D & E* × 278 ; *F—I* × 133.

11. **Spirogyra biformis Jao** Jao. New Zygnemataceae collected in China, *American Journal of Botany*, Vol. 23, No. 1, 1936, p. 54, Figs. 4 & 5.

Lat. cell., 40-46 μ ; long. cell., 46-165 μ ; crass. zygosp., 40-46 μ ; long. zygosp., (50-) 60-90, average 60-69 μ.

Habitat :—In road-side puddles along with *Spirogyra* sp. Hoshangabad.

The scalariform conjugation, though rare in the Chinese form, is quite common in this alga.

12. **Spirogyra Fullebornei Schmidle forma Rao.** Rao, *op. cit.*, 1937, p. 279, Fig. 4, *B*.

Lat. cell., 50-52 μ ; long. cell., 66-148 μ ; crass. zygosp., 42-56 μ, average 48-52 μ ; long. zygosp., 66-85 (-92) μ ; crass. zygosp. subspheric., 42-60 μ.

Habitat :—In a rain-water pool, Hoshangabad.

13. **Spirogyra columbiana Czurda** Czurda, *op. cit.*, 1932, p. 190, Fig. 199.

Forma:

Lat. cell., 52-60 μ ; long. cell., 50-150 μ ; crass. zygosp., 44-56, average 50 μ ; long. zygosp., 68-90, average 75 μ.

Habitat :—In a pond, Katni.

The form differs from the type in the cells being slightly broader and the zygospores being sometimes longer.

14. **Spirogyra neglecta (Hassall) Kützing** Czurda, *op. cit.*, 1932, p. 191, Fig. 200.

Lat. cell. 60-66 μ ; long. cell., 82-171 μ ; crass. zygosp., 60-68 μ ; long. zygosp., 81-100 (-113) μ.

Habitat :—In a pond, Katni.

Forma Rao. Rao, *op. cit.*, 1937, p. 282, Fig. 5, *B*.

Lat. cell., 60-66 μ ; long. cell., 49-120 μ ; crass. zygosp., 52-60 μ ; long. zygosp., 70-92 (-98) μ.

Habitat :—In Budagar lake, along with *Spirogyra ternata* forma, Durie.

15. **Spirogyra nitida (Dillwyn) Link.** Borge & Pascher in Pascher's Süsswasserflora, Heft 9, 1913, p. 26, Fig. 37 ; Jao, Studies on the Freshwater Algae of China, I, Zygnemataceae from Szechwan, *Sinensia*, Vol. 6, No. 5, 1935, Pl. VI, Figs. 70 & 71.

Lat. cell., 68-82, average 82 μ ; long. cell., 110-720 μ : crass. zygosp., 66-75 μ ; long. zygosp., 95-115 μ.

Habitat :—In a rain-water pond, Hoshangabad.

16. ***Spirogyra setiformis*** (**Roth.**) **Kützing** Czurda, *op. cit.*, 1932, p. 192, Fig. 202.

Forma

Lat. cell., 85-95 (-100) μ ; long. cell., 100-148 μ : crass. zygosp., 60-73, average 66 μ ; long. zygosp., 90-132, average 105-115 μ.

Habitat :—In puddles on the Hoshangabad rock-quarry, along with a sterile species of *Oedogonium*.

This form possesses narrower cells on the average and some times longer zygospores.

Forma

Lat. cell., 95-102 μ; long. cell., 82-320 μ; crass. zygosp., 82-89 μ; long. zygosp., 105-138 (-150) μ.

Habitat:—In a stagnant puddle on the Hoshangabad rock-quarry along with *Spirogyra submaxima* var. *lamellata, S. dubia*, *Sirogonium ventersicum* forma, *Aulosira Fritschii, Oedogonium* sp., *Anabaena* sp. and several others.

The zygospores in this form are sometimes much longer.

17. ***Spirogyra pseudoneglecta*** **Czurda** Czurda, *op. cit.*, 1932, p. 194, Fig. 204.

Forma

Lat. cell., 46-48μ; long. cell., 50-116 μ; crass. zygosp., 43-46μ; long. zygosp., 60-72 μ; crass. cell. fructif., upto 46 μ.

Habitat:—In a pool (Madhav Tal) on hills along with *Spirogyra dubia* var. *polymorphis*, Jubbulpore.

This form differs from the type in the narrower cells and smaller zygospores.

18. ***Spirogyra verruculosa*** **Jao** Jao, *op. cit.*, 1936, p. 59, Figs. 32 & 33.

Forma (Fig. 3, *A*).

Lat. cell., 106-120 μ; long. cell., 120-130μ; crass. zygosp., 69-90 μ; long. zygosp., 102-132 μ.

Habitat :—In a pool (Deva Tal) along with a sterile species of *Spirogyra* and *Anabaena*, Jubbulpore.

The form differs from the type in the chloroplasts being 5-8 and the zygospores being smaller with frequently more or less drawn out ends.

19. **Spirogyra anomala Rao**   Rao, *op. cit.* 1937, p. 284. Fig. 6, *D* & *E*.

Forma

Lat. cell., 65-96 μ; long. cell., 96-237 μ; crass. zygosp., 55-78 μ; long. zygosp., 89-122 μ.

Habitat:—In a rain-water pool, by the side of the road leading to Bombay from Hoshangabad.

The form differs from the type in having much narrower cells and zygospores which are narrower and frequently shorter.

20. **Spirogyra Malmeana Hirn**   Czurda, *op. cit.*, 1932. p. 202, Fig. 216.

Var. *verrucosa* var. nov.   (Fig. 3, *B* & *C*).

Vegetative cells thrice as long as broad; end-walls plane; chloroplasts 4-6. Conjugation scalariform; fructifying cells unswollen. Zygospores ellipsoidal with rounded or pointed ends; exospore thin, smooth and hyaline; mesospore thick, yellowish-brown, finely verrucose and marked by a continuous net-work with large meshes.

Lat. cell., 108-126μ; long. cell., 120-330 μ; crass. zygosp., 89-100μ; long. zygosp., 115-165μ.

Habitat:— In a pool (Deva Tal) on hills, along with sterile filaments of *Oedogonium* and *Spirogyra*, Jubbulpore.

The variety agrees with the type in the plane end-walls, scalariform conjugation and ellipsoidal zygospores with a thin, smooth and colourless exospore, and a thick brown mesospore, but differs from the same in the much broader filaments, and in having more chloroplasts and markedly bigger zygospores, the latter possessing a finely verrucose mesospore with a more regular net-work.

21. **Spirogyra submaxima Transeau**   Czurda, *op. cit.*, 1932, p. 206, Fig. 221.

Var. *lamellata* var. nov. (Fig. 3, *D*).

Vegetative cells upto six times as long as broad; end-walls plane; chloroplasts 6-8. Conjugation scalariform; fructifying cells unswollen. Zygospores lens-shaped, spherical in one view and ellipsoidal in the other; exospore thin, smooth and colourless; mesospore thick, lamellate and yellowish-brown; endospore indistinct.

Lat. cell., 76-79 μ; long. cell., 151-466 μ; crass. zygosp. spheric., 67-83 μ; lat. zygosp. lens., 50-60 μ; crass. mesospore 3-5 μ:
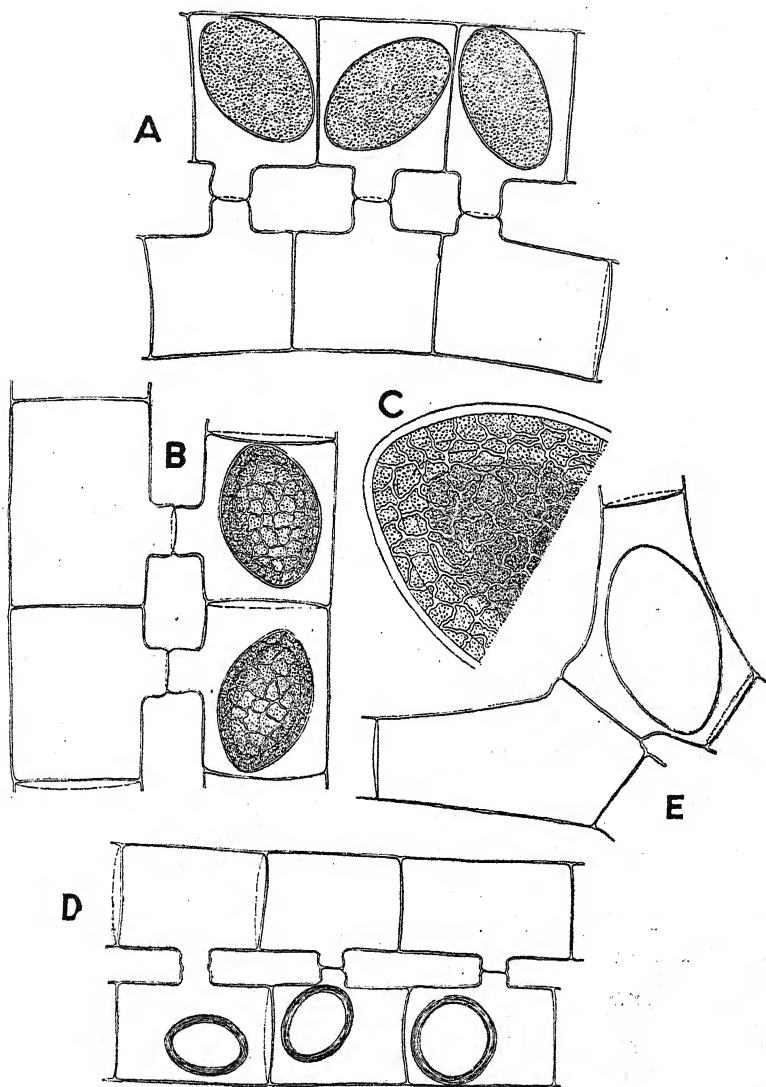
Fig. 3. *A*—Conjugating filaments of *Spirogyra verruculosa* Jao forma with zygospores; *B* — Conjugating filaments with zygospores; *C* — a highly magnified zygospore showing the surface sculpture (edge-view) of *Spirogyra Malmeana* Hirn var. *verrucosa* var. nov.; *D* — Conjugating filaments of *Spirogyra submaxima* Transeau var. *lamellata* var. nov. with zygospores; *E* — Conjugating filaments of *Sirogonium floridana* Transeau forma with a zygospore. *A* & *B* ×133; *C* ×278; *D* & *E* ×133.

Habitat :- In a puddle on the Hoshangabad rock-quarry, along with *Spirogyra setiformis* forma, *S. dubia* forma, *Sirogonium ventersicum* forma, *Aulosira Fritschii*, *Anabaena* sp., *Oedogonium* sp. and several others.

The variety agrees with the type in the scalariform conjugation and lenticular zygospores with a thick, smooth and yellowish-brown mesospore. But it differs from the same in having on the average narrower filaments without any mucilaginous envelope and lesser number of chloroplasts, in the fructifying cells being never swollen on the side of the conjugation canal, and in the mesospore of the zygospore being lamellated.

(*b*) **Mougeotiaceae.**

Genus *Mougeotia* Agardh.

22. *Mougeotia recurva* (*Hassall*) de Toni Czurda, *op. cit.*, 1932, p. 67, Fig. 41.

Lat. cell., 13-15 μ; long. cell., 115-231 μ; crass. zygosp. spheric., 25-26 μ.

Habitat :- In a pool (Kola Tal) on hills, along with *Mougeotia tenuis* and *Spirogyra longata* forma, Jubbulpore.

23. *Mougeotia tenuis* (*Cleve*) Wittrock Czurda, *op. cit.*, 1932, p. 81, Fig. 66; Rao, *op. cit.*, 1937, Fig. 7, *E-G*.

Lat. cell., 13-14 μ; long. cell., 130-198 μ; crass. zygosp., 19-26 μ; long. zygosp., 30-31 μ.

Habitat :— In a pool (Kola Tal) on hills, along with *Spirogyra longata* forma and *Mougeotia recurva*, Jubbulpore.

Genus *Sirogonium* Kützing.

24. *Sirogonium floridana* Transeau [=*Spirogyra floridana* Transeau] Czurda, *op. cit.*, 1932, p. 145.

Forma (Fig. 3, *E*).

Vegetative cells 3-4 times as long as broad; end-walls plane; chloroplasts 5-8. Conjugation scalariform; fructifying cells inflated. Zygospores ellipsoidal; exospore, thin, smooth and hyaline; mesospore thin, smooth and yellow.

Lat. cell., 66-82, average 66 μ; long. cell., 200-275μ; crass. zygosp., 82-100, average 89 μ; long. zygosp., 115-165, average 130 μ; crass. cell. fructif. 90-150 μ.

Habitat :—In a puddle near the rock-quarry, Hoshangabad.

The form differs from the type in the presence of broader cells, 5-8 chloroplasts and sometimes longer zygospores.

25. **Sirogonium ventersicum Transeau** Transeau, Tiffany, Taft and Li, New species of Zygnemataceae, *Transactions of the Microscopical Society,* Vol. LIII, No. 3, 1934, Pl. XXII, Fig. 65.

Forma

Lat. cell., 66-70 μ ; long. cell., 122-171 μ ; crass. zygosp., 70-78 μ ; long. zygosp., 102-151 μ ; crass. cell. f r u c t i f ., 100-148 μ.

Habitat :—In a stagnant puddle on the Hoshangabad rockquarry, along with *Spirogyra submaxima* var. *lamellata, S. setiformis* forma, *S. dubia* forma, *Aulosira Fritschii, Anabaena* sp., *Oedogonium* sp., and several others.

This form possesses narrower zygospores that are sometimes shorter.

This investigation was carried out in the Botanical Laboratory of the Benares Hindu University while the writer was a Research Scholar there. The writer takes this opportunity to express his great indebtedness to Professor Y. Bhâradwâja for his kind guidance and criticism during the preparation of this paper.

ON A NEW SPECIES OF *DADOXYLON*, *D. DECCANI*, SP. NOV., FROM THE DECCAN INTERTRAPPEAN SERIES

BY

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Contents

Introduction

The solitary block of gymnospermous wood here described belongs to the Central Museum, Nagpur where it had been preserved probably since the time of the late Rev. S. Hislop and it was through the kindness of Mr. E. A. D'Abreu, formerly curator of the museum, that the author was able to borrow it for investigation.

Only the secondary wood is available. The geological age is not known but the entries in the museum register indicate that the specimen was collected in the Chhindwara district of the Central Provinces. It may therefore perhaps be considered to belong to the Deccan Intertrappean series which according to Prof. Sahni[1] is probably of early Tertiary age. So far as I know no coniferous woods have yet been described from the Deccan Intertrappean Series although Professor Sahni[2] has described

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[1] Sahni, B., Current Sci. Vol. III, No. 4, pp. 134-136, (1934).

[2] Sahni, B., Mem. Geol. Sur. Ind., Palaeont. Indica, N. S., Vol. II, pp. 79-97, (1931).

three genera of seed-bearing cones of conifers from this Series. The present discovery suggests a careful search for other coniferous remains in these beds.

Description

A well preserved piece of secondary wood, 10 cm. long, 7·5 cm. thick (Pl. XIV, fig. 1), far away from the pith as indicated by the flat arcs of the growth rings (Text-fig. 1).
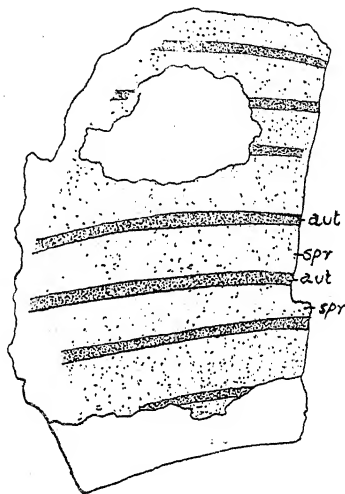
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Fig. 1.—Type specimen. Diagram of a transverse view showing flat arcs of growth-rings (× 2/3).

Microscopically the growth-rings are fairly distinct (Pl. XIV, fig. 2). They can also be made out with the naked eye, as the autumn wood is darker in colour than the spring wood. The former in average width measures 1 cm., the latter 5 mm.

The diameter of the entire stem is roughly calculated to be about 23 inches on the basis of the arcs of the growth rings.

In transverse section the tracheids are more or less isodiametric. It is seen that in the wood of each season some rows of tracheids—particularly those adjacent to the medullary rays—are comparatively broader than others (Pl. XIV, figs. 2, 3 x). This feature is also marked in the tangential section of the wood, (Pl. XV, fig. 5 x).

The medullary rays count from 2 to 49 cells in height (Pl. XV, fig. 5) and the end walls of the cells are either at right angles or oblique to the tracheids. They are often separated from one another by a single or double row of resiniferous tracheids (Pl. XIV, fig. 4; Text-fig. 2 R. tr.).
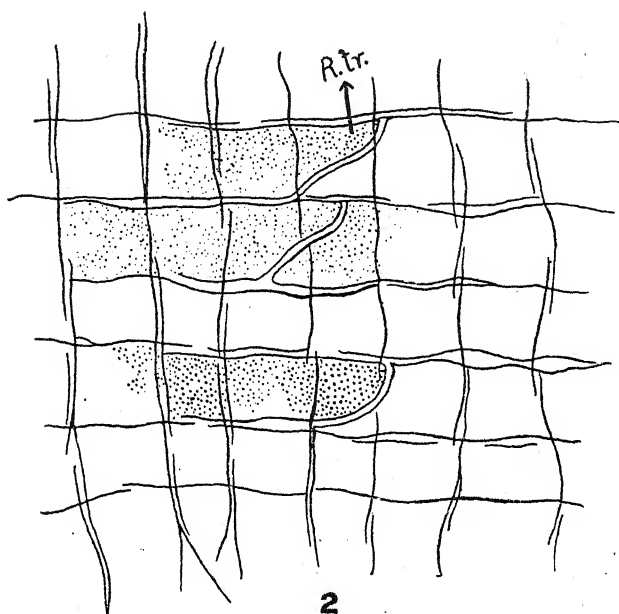
Fig. 2.—Part of the radial section. *R. tr.* resiniferous tracheids in the medullary rays (× 340).
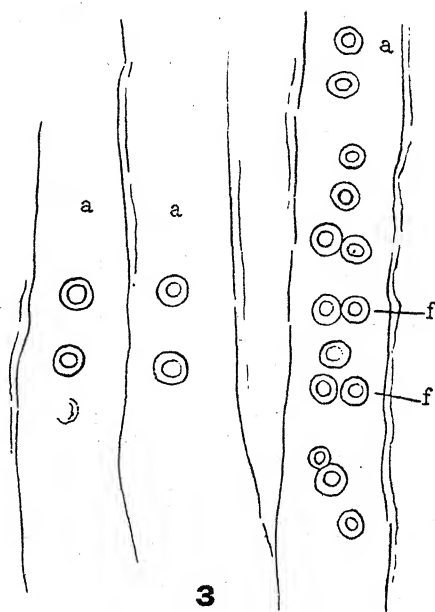
Fig. 3.—Part of the radial section showing bordered pits (× 400).

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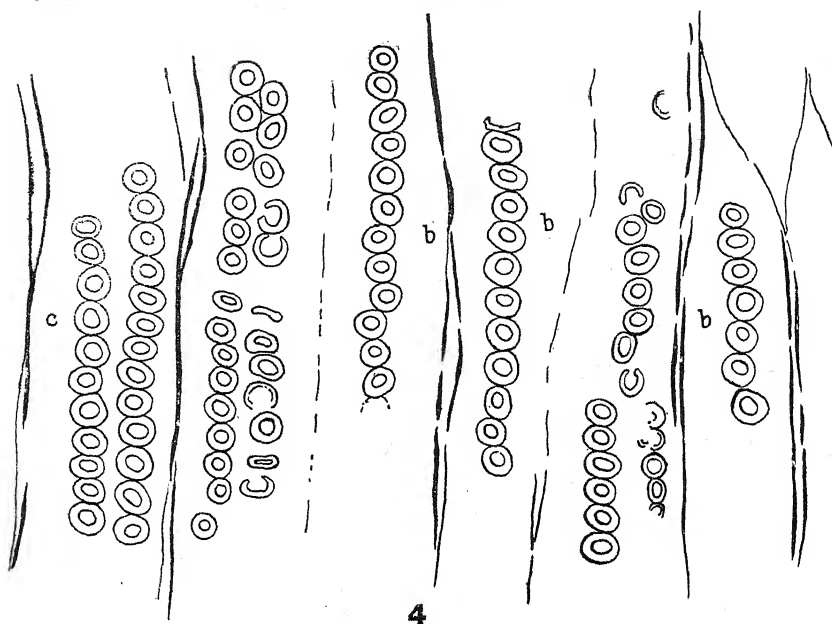
Fig. 4.—Part of the radial section showing contiguous nature of the bordered pits (× 400).

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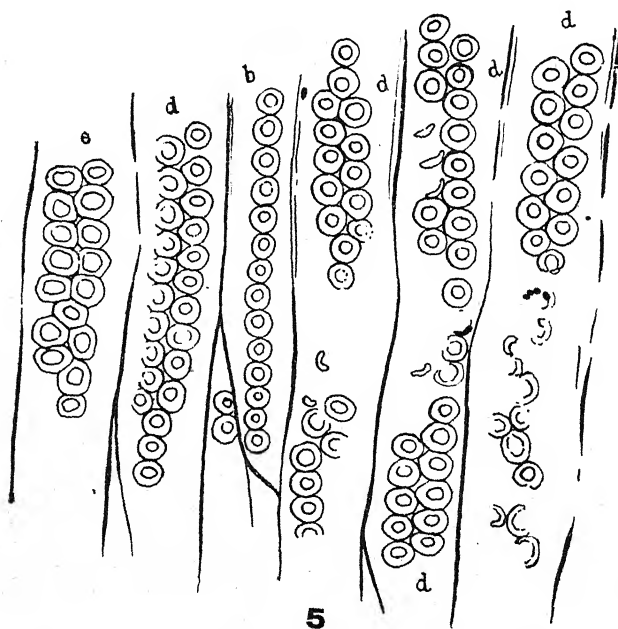
Fig. 5.—Bordered pits with varying degrees of contiguosity as seen in the radial section (× 400).

The pits which occur on the radial walls of the tracheids show considerable variation in their arrangement. In some tracheids they lie in a single row quite separate from each other (Text-fig. 3a) and in others they are contiguous either in one row (Pl. XV, fig. 6; Text-figs. 4, 5b) or in two separate rows (Pl. XV, fig. 6; Text-fig. 4c).

Sometimes the two rows may lie in contact with each other with various degrees of compression, the pits lying alternately (Pl. XV, fig. 7; Text-figs. 5 and 6d). This compression in some
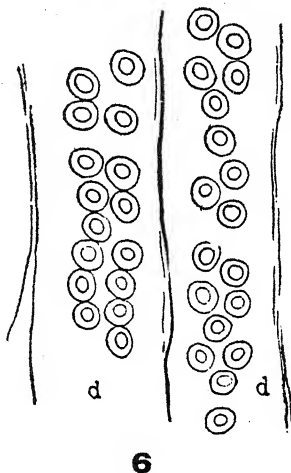
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Fig. 6.—Slightly compressed bordered pits as seen in the radial section (×400).
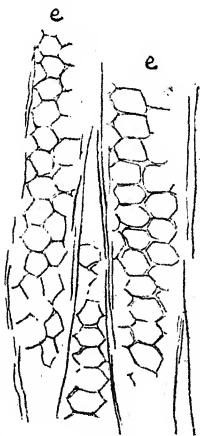
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Fig. 7.—Part of the radial section showing contiguous hexagonal pitting (×327).

6

cases has resulted in the formation of contiguous hexagonal pitting (Pl. XV, fig. 8; Text-figs. 5 and 7e).

A feature unusual for woods of the Araucarian type is that a few tracheids in this specimen show the pits lying exactly and distinctly opposite to each other (Text-figs. 3 and 8f) and in some cases even with a common wall (Fig. 7g).

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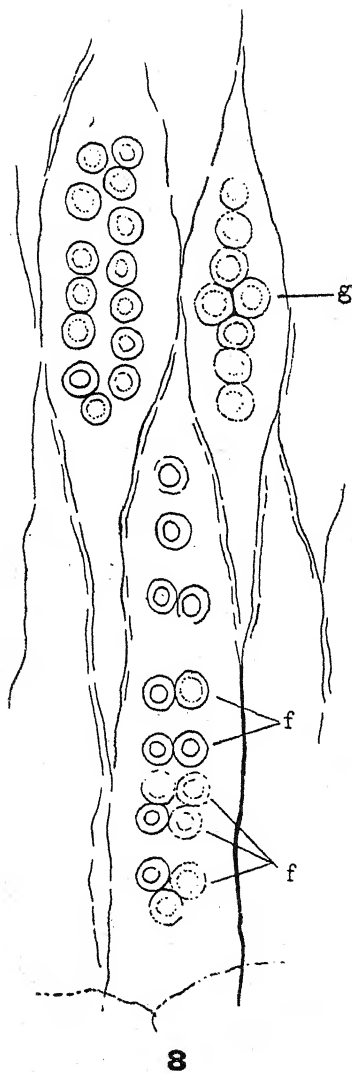
Fig. 8.—Part of the radial section showing bordered pits lying opposite to each other (×407).

In each field there are usually 2-4, sometimes 6 oval narrowly bordered pits of which the large circular or elliptical, usually oblique pore is often seen preserved (Pl. XV, figs. 9 & 10; Text-figs. 9 & 10).

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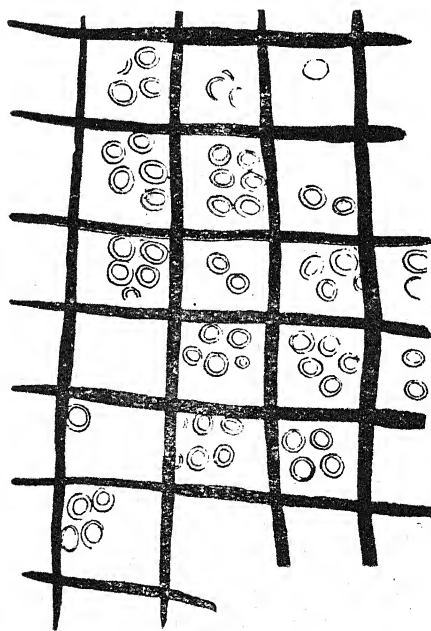
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Figs. 9 ($\times$ 300) and 10 ($\times$ 400). Pits in the field (Type-specimen).

Average radial diameter of widest spring tracheids $= 80\mu$

   Do.   do.   narrowest autumn   ,, $= 17\mu$

Height of a vertical row of 10 radial pits $= 96 \cdot 4\mu$

Height of medullary rays (average 50 counts) $= 18$ cells.

Average dimensions of medullary ray cells $= 44 \times 33\mu$

Diagnosis

Growth rings well marked, resiniferous tracheids occur mixed with medullary rays, radial pits round or elliptical, 1-2 seriate, bordered, usually alternate, sometimes contiguous and hexagonal due to contact, sometimes also opposite; often separate in one or two rows and sometimes contiguous even when in a single row. Pore large and circular, rims of Sanio absent. Medullary rays mostly uniseriate, rarely biseriate in the middle, 2-49 cells high, average height 18 cells, cells in tangential section of the wood nearly isodiametric or slightly broader than high. End walls of the medullary ray cells either at right angles or oblique to the tracheids. Pits in the field 1-6, bordered, usually round, sometimes elliptical, pore when preserved usually round, sometimes elliptical.

Locality.—Chhindwara district, C. P., India.

Horizon—Unknown, possibly Intertrappean Series of the Deccan.

Reg. No.—F/250, Central Museum, Nagpur, C. P.

Systematic position

As stated by Professor Seward[1], it is not easy to assign any fossil wood with Araucarian pitting either to the Cordaitales or to the Araucarineae merely on the basis of the age of the strata, because we neither definitely know the uppermost limits of the Cordaitales nor the lower most limits of the Araucarineae. It is moreover probable that the two families might have existed side by side for a considerable time. Hence the use of the terms *Araucarioxylon* or *Cordaioxylon* on the basis of the geological age alone, particularly in a case like this, where the material is fragmentary, may be open to objection.

Prof. Seward[2] suggests the term *Dadoxylon* for all woods showing Araucarian or Cordaitalean characters of which the actual affinities are unknown.

As there is absolutely no evidence of Cordaitales persisting into the Cretaceous or Tertiary, the specimen is referred to the artificial genus *Dadoxylon* (*Araucarioxylon*), any further clue to

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[1]Seward, A. C., Fossil Plants, III, pp. 249-250, (1917).
[2]*Ibid.* p. 249, (1917).

the affinity on the basis of the pith and primary xylem being entirely out of question in this particular case.

Comparisons

The wood shows considerable differences from other species. As *Dadoxylon* (*Mesopitys*) *tchihatcheffi*[1], the only northern species so far known to show growth rings, differs from the present species in having markedly low medullary rays[2], the comparison of the wood is confined to the southern species.

(*a*) *D. Zalesskyi* Sahni[3] differs from this species in having 1-5 rows of pits, low medullary rays which are always uniseriate, and pits in the field devoid of a border.

(*b*) *D. Bakeri* Seward and Walton[4], a Falkland wood ; *Dadoxylon sp.* Warren[5] ; *Dadoxylon sp.* Walton[6], both the latter from South Africa and another Falkland wood referred by Halle to *D. angustum* Felix[7], which have all been put under the name *D. Arberi* by Walton, differ in several respects from this species. The comparatively low medullary rays (1-16 cells high in *D. Bakeri*, 1-20 in *Dadoxylon sp.* Walton and 1-25 in *D. angustum*) alone would serve to distinguish the present species. The uniseriate to triseriate nature of the pits found usually in all these species is again different from the uni- or biseriate nature of pits seen in the present species.

(*c*) The new species also differs from a wood from Sugar Loaf Range in the Newcastle Coal field assigned by Sahni and Singh to *D. Arberi*[8] and later renamed by Sahni as *D. Kräuseli*, sp. nov.[9] The presence of rims of Sanio, the comparatively low medullary rays (1-20 cells high) and a fairly large number of pits in the field in *D. Kräuseli* (2-10) are features of marked difference from those of the new species.

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[1]Frentzen, K., Centralbl. f. Min. etc., Abt. B. No. 11, pp. 617-624, (1931).

[2]Seward, A. C., Fossil Plants, III, p. 296, (1917).
[3]Sahni, B., Rec. Geol. Surv. India., LXVI, p. 421, (1933).
[4]Seward, A.C. & Walton, J. Quart. Jour. Geol. Soc., LXXIX, p. 327, (1923).
[5]Warren, E., Ann. S. Afr. Mus., II, pt. III, (1912).
[6]Walton. J., Ann. S. Afr. Mus. XXII., p. 2, (1925).
[7]Halle, T. G., Bull. Geol. Inst. Univ. Upsala. XI., p. 68, (1911).
[8]Sahni, B. & Singh, T.C.N., Jour. Ind. Bot. Soc. V., No. 3, pp. 103-112, (1926).
[9]Sahni, B., Rec. Geol. Surv. India. LXVI, p. 424, (1933).

(d) *D. Lafoniense* Halle[1], a Falkland wood described by
Prof. Halle, resembles this species in having usually
biseriate pits but differs considerably in its unusually
low uniseriate medullary rays like *D. Zalesskyi.* The
presence of secretary sacs in *D. lafoniense* is again
a point of difference.

(e) *D. indicum* Holden[2], a species from the palaeozoic beds
of India, resembles this new species in having 1—2
rows of pits but differs in having low uniseriate
medullary rays and pits in the field having a tendency
to fuse and unite to form a single pit.

(f) *D. bengalense* Holden[3], a species from the same locality
as *D. indicum* Holden, is different in having 2—7 pits
forming a group and in having always uniseriate
medullary rays whose heights may be 1—20 cells.

(g) *D. (Arauc.) rajmahalense* Sahni[4], a species from the
Rajmahal Hills, India, differs from this species in
having 2—3 seriate pits and uniseriate medullary rays.
The number of cells forming the height of the medull-
ary rays too is much smaller, being only 1—20.

(h) *Dadoxylon* ∝ Sahni[5] and *Dadoxylon* β Sahni[6] are again
different, as the former is characterised by uniseriate,
low medullary rays and the latter in having 2—3
seriate pits and uniseriate medullary rays which are
low. The growth rings too are not clearly marked in
either of the species.

(i) *D. parbeliense* Rao[7], a Lower Gondwana wood contained
in a sphaerosiderite from the Ranigunj Coalfield is
also different in having 1-5 seriate pits, low medullary
rays (1-24 cells high) and 8-9 pits spanning a field.

(j) *D. teilhardi* Sze[8], a species described from the Upper
Permian of China and belonging to the Angara flora,
resembles this species in having 3-6 pits in the field
and usually biseriate pits though triseriate ones also
exist, but the presence of uniseriate and very low
medullary rays (1-6 cells high) are features which
keep it distinct from the new species.

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[1]Halle, T. G., Bull. Geol. Inst. Univ. Upsala. XI. p. 64, (1911).
[2]Holden, R., Ann. Bot. XXXI. p. 318, (1916).
[3]Holden, R., Ann. Bot. XXXI. p. 322, (1916).
[4] [5], [6]. Sahni, B., Pal. Ind. N. S. Vol. XI. pp. 69, 71, 72 respectively, (1931).
[7]Rao, H. S., Rec. Geol. Surv. Ind. LXIX. pp. 174-183, (1935).

[8]Sze, H. C., Bull. Geol. Soc. China, XIII, No. 4, (1934).

(k) D. rhodeanum Göppert[1], a Palaeozoic wood widely distributed in China, particularly in Shansi, and determined and described by Gothan and Sze, differs from the present species in having 2-4 rows of pits, rather low medullary rays (3-20 cells high) and in the entire absence of annual rings.

In the circumstances the present wood seems to be different from any other species hitherto known, and there being none so far described from the Intertrappean beds of India, it is proposed to give it a new name : *Dadoxylon Deccani*.

Discussion

The present wood exhibits, in addition to *Dadoxylon* characters, also the interesting feature of the combination of opposite pits. This opposite nature of pits may not necessarily be confined to the Abietineae and other coniferous families but may also be found in the araucarian members. Such a case has as well been recorded by Miss Holden[2] in the genus *Metacedroxylon araucarioides* which in addition to other Dadoxylon characters also shows opposite pits. This she definitely concludes to be an araucarian genus and thinks it to be a connecting link between the Araucarineae and the Abietineae. Such a combination of alternate and opposite pits she has also recorded in *Araucarioxylon* sp. Holden from the Cretaceous lignites of Cliffwood, New Jersy. Prof. Seward has also adopted the name *Dadoxylon*[3] for this wood because the characters as a whole are consistent with that designation.

Another noteworthy feature in the present wood, in addition to the combination of two types of pits, is that its age is not Cretaceous. As we feel convinced that the Intertrappean Series of the Deccan are Tertiary[4] particularly in view of the new discoveries from these beds of *Nipadites*, a fossil fruit genus of Palms eminently characteristic of the Eocene[5], it may be quite plausible to conclude that the combination of the two characters existed even till a later stage than the one recorded by Miss Holden.

Summary

A piece of secondary wood has been described here which belongs probably to the Deccan Intertrappean Series. The name *Dadoxylon Deccani* sp. nov. has been given to it. The wood shows opposite pits in addition to the contiguous hexagonal pitting

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[1]Gothan, W., und Sze, H. C., Mem. Nat. Res. Inst. Geol. No. 13, pp. 87-103, Nanking, (1933).

[2]Holden, R., Ann. Bot., XXVII, pp. 538-539, (1913).

[3]Seward, A. C., Fossil Plants., IV. p. 183, (1919).

[4]Sahni, B., Current Sci., Vol. III, No. 4, pp. 134-136, (1934).

[5]Sahni, B., and Rode, K. P. Proc. National Acad. Sci. Ind. 7 (2-3), Allahabad.

characteristic of the Araucarineae. This combination of two characters indicates that the wood may be considered as a connecting link between the Araucarineae and the Abietineae.

The writer is greatly indebted to Prof. Sahni for his constant help, valuable suggestions and criticisms during the progress of this work. My thanks are also due to my friend Dr. K. M. Gupta for some suggestions in this connection and to the Nagpur University for getting some sections prepared from the fossil.

Bibliography

FRENTZEN, K.—' Die palaeogeographische Bedeutung des Auftretens von Zuwachszonen bei Hölzern der Sammelgattung *Dadoxylon*', Centralbl. f. Min. etc., Abt. B, No. 11, pp. 617-624, (1931).

GOTHAN, W. UND Sze, H. C.— ' Ueber Fossile Hölzer aus China '. Mem. Nat. Res. Inst. Geol. No. 13, pp. 87-103, Nanking, (193).

HALLE, T. G.—' On the geological structure and history of the Falkland Islands ', Bull. Geol. Inst. Univ. Upsala, XI, (1911).

HOLDEN, R.—' On the anatomy of two Palaeozoic stems from India ', Ann. Bot., XXXI, pp. 315-326, (1916).

————————' Contributions to the anatomy of Mesozoic Conifers No. 1. Jurassic Coniferous woods from Yorkshire. pp. 538-546, Ann. Bot., XXVII, (1913).

RAO, H. S.—' On a sphaerosiderite, containing a new species of *Dadoxylon* from the Lower Gondwana Coal Measures of India '. Rec. Geol. Surv. Ind., LXIX, pp. 174-183, (1936).

SAHNI, B.—' Revision of Indian Fossil Plants ', Pt. II, Coniferales, (b. Petrifactions). Pal. Ind., N. S., XI, pp. 69-97, (1931).

———————' *Dadoxylon Zalesskyi*, A new species of Cordaitean trees from the Lower Gondwanas of India '. Rec. Geol. Surv. Ind., LXVI, pt. IV, p. 421, (1933).

———————and Singh, T.C.N.—' On some specimens of *Dadoxylon* Arberi (Sew.) from Queensland and New South Wales. Jour. Ind. Bot. Soc., V., No. 3, pp. 103-112, (1926).

———————'The Deccan Traps : Are they Cretaceous or Tertiary ?' Current. Sci. Vol. III, No. 4, pp. 134-136, (1934).

———————and RODE, K.P.—' Fossil plants from the Deccan Intertrappean beds at Mohgaon Kalan (C. P.) with a note on the geological position of the plant-bearing beds.' Proc. National Acad. Sci. Ind. 7 (2-3), Allahabad.

SEWARD, A. C.—'Fossil Plants', III, (1917).

————————'Fossil Plants', IV, (1919).

————————and Walton, J.—'On some fossil plants from the Falkland Islands'. Quart. Jour. Geol. Soc., LXXIX, pp. 313-333, (1923).

SZE, H. C.—'On the occurrence of an interesting fossil wood from Urumchi (Tihua) in Sinkiang'. Bull. Geol. Soc. China., XIII, No. 4, (1934).

WALTON, J.—'On some South African fossil woods'. Ann. S Afr. Mus. XXII, (1925).

WARREN.—'On some specimens of fossil woods in the Natal Museum'. Ann. S. Afr. Mus., II, Pt. 3, (1912.)

Explanation of Plates XIV and XV

Fig. 1.—*Dadoxylon Deccani,* sp. nov. Type specimen ($\times$ ca 1/10).

Fig. 2.—Type specimen. Cross section of stem showing a growth-ring ($\times$ 20) (rows of tracheids marked$\times$ are comparatively broader than others).

Fig. 3.—Type specimen. Cross section of the stem showing broad rays of tracheids adjacent to the medullary rays ($\times$ 150).

Fig. 4.—Type specimen. Radial section of the stem showing resiniferous tracheids in the medullary rays ($\times$80).

Fig. 5.—Type specimen. Tangential section to show the medullary rays ($\times$ 320). The tracheids marked $\times$ are broader than others.

Fig. 6.—Type specimen. Radial longitudinal section showing contiguous pits in one row and contiguous pits in two separate rows ($\times$ 320).

Fig. 7.—Type specimen. Radial longitudinal section showing slightly compressed and alternate pits ($\times$320).

Fig. 8.—Type specimen. Radial longitudinal section showing contiguous and hexagonal pitting ($\times$320).

Figs. 9 & 10.—Type specimen. Radial longitudinal section showing pits in the field ($\times$ 250).
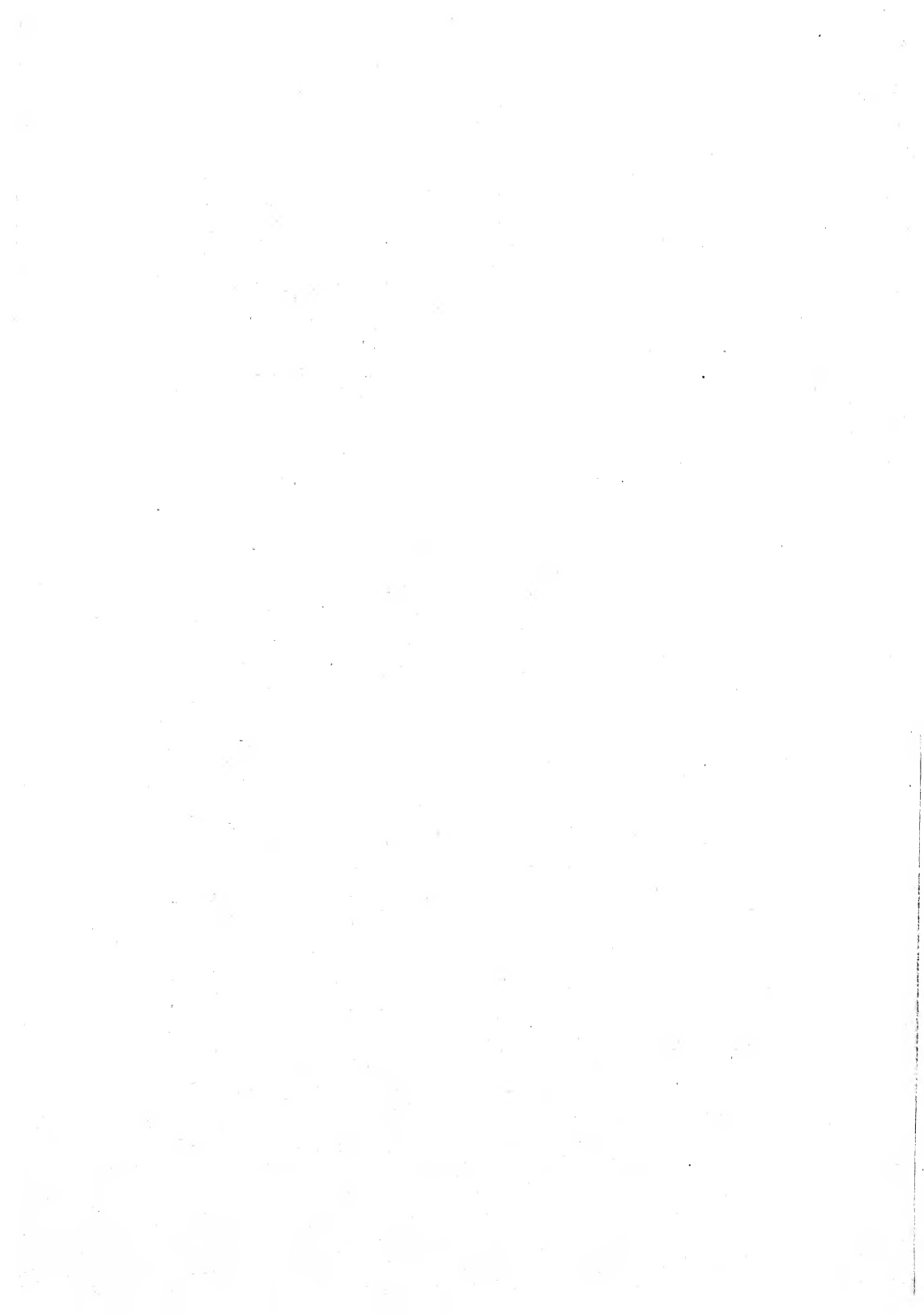
Fig. 1 (x Ca $\frac{1}{10}$)
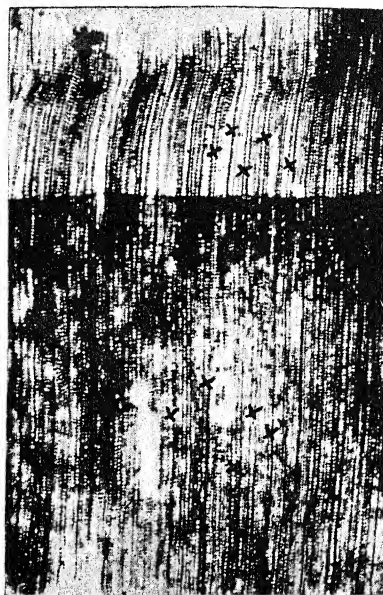
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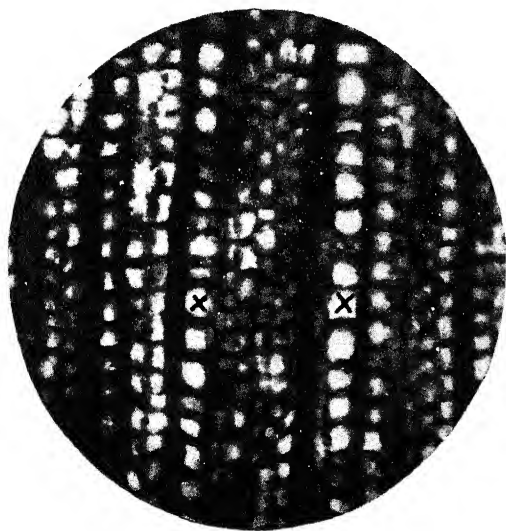
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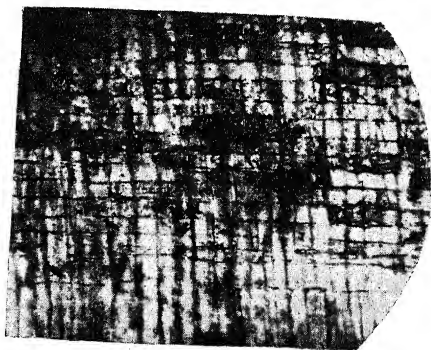
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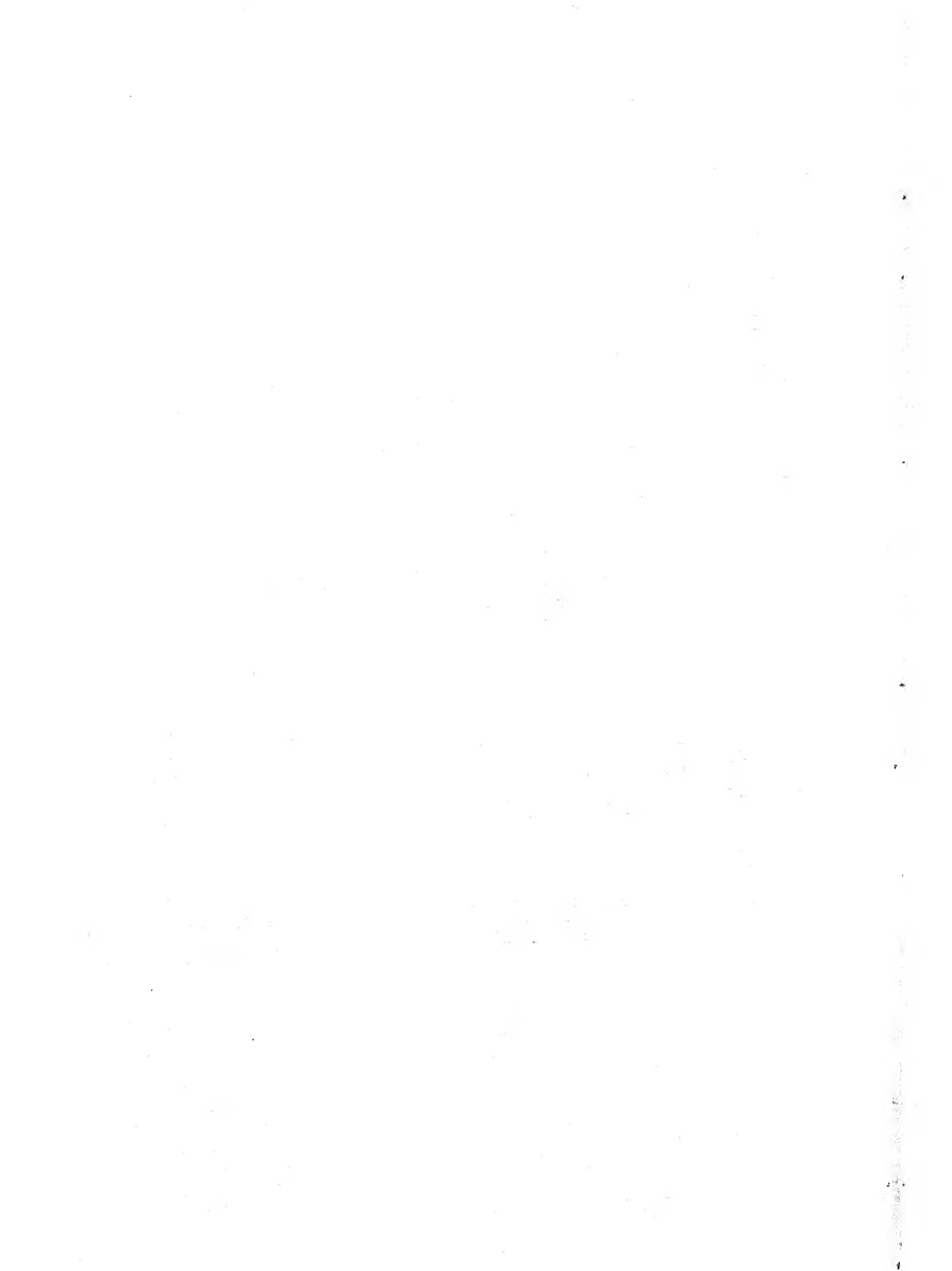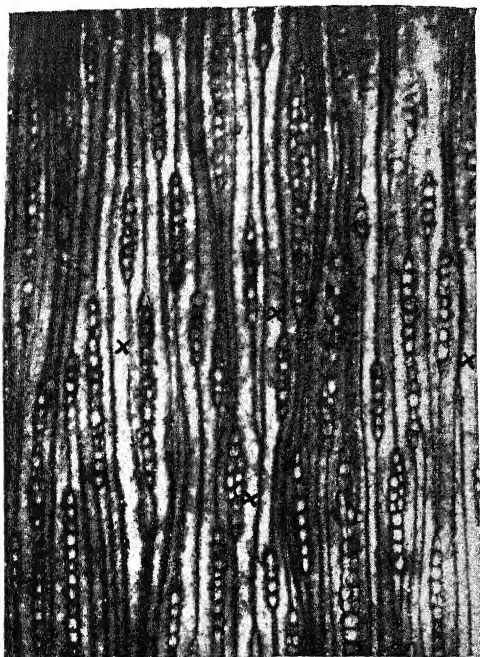
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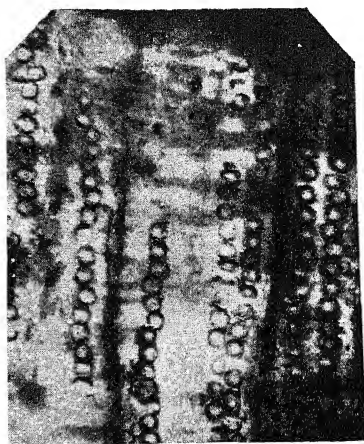
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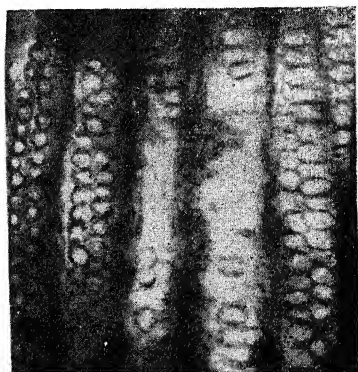
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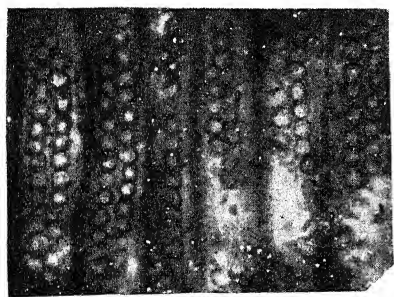
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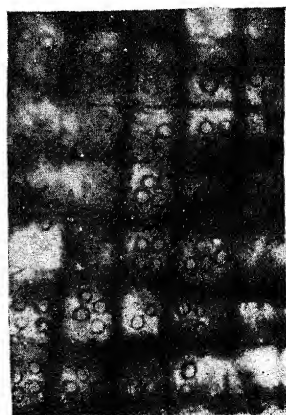
Fig. 10 (x 250)

THE ZYGNEMOIDEAE OF
THE UNITED PROVINCES, INDIA—II*

BY

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The present communication forms the second [1] of the series in which it has been proposed to record the Zygnemoideae of the United Provinces. Most of the forms that are described in this paper have been collected from Gorakhpur and its environs during the last two years. Four forms collected from Benares and one from Sakaldiha have also been included. With the exception of *Zygnema globosum* Czurda and *Spirogyra varians* (Kütz.) Czurda all the plants were got from rice fields. In all twenty-five forms have been recorded, and out of these six species, four varieties and eleven forms are new.

SYSTEMATIC ENUMERATION OF THE SPECIES OBSERVED.

(a) Zygnemaceae.
Genus *Zygnema* Agardh.

1. *Zygnema globosum* Czurda Czurda, in Pascher's Süsswasser-flora, Mitteleuropas, Heft 9, 1932, p. 109, Fig. 110.

Forma

Lat. cell., 25-31 μ; long. cell., 30-40 μ; crass. zygosp. spheric., 42.9-45 μ; crass. zygosp. oval., 36.3-42.9 μ; long. zygosp. oval., 42.9-44 μ; scrobiculations 3-4μ broad and 1-1.5 μ apart.

Habitat :— In a rain-water pool, along with sterile filaments of *Spirogyra* and *Oedogonium*, Gorakhpur. (October 20, 1936.)

This form differs from the type in possessing smaller zygospores. It also has occasionally broader cells.

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*From the Department of Botany, Benares Hindu University.

[1] Bhashyakarla Rao, C., "The Zygnemoideae of the United Provinces, India—1," *Jour. Ind. Bot. Soc.*, 1937, Vol. XVI, No. 5, pp. 269-288.

2. **Zygnema gorakhporense sp. nov.** (Fig. 1, A. & B).

Vegetative cells upto four times as long as broad; conjugation scalariform; zygospore formation in the conjugation-canal which becomes bulged out. Zygospores globose to sub-globose; exospore thin, smooth and hyaline; mesospore, thick, lamellated, blue with broad scrobiculations.

Lat. cell., 23.1-26.4 μ; long. cell., 66·82.5 μ; crass. zygosp. spheric., 36.3-42.9 μ; crass. zygosp. sub-spheric., 30-35 μ; long. zygosp. sub-spheric, 36.3-42.9 μ; scrobiculations, 4 μ broad and 1-3 μ apart.

Habitat:—Along with *Bulbochaete Bharadwajai* sp. nov., Gorakhpur. (October 8, 1936.)

The alga belongs to the Section 'Pectinata' of the genus on account of the formation of zygospores in the conjugation-canal (Czurda, *op. cit.*, 1932, p. 99). The alga is comparable to *Zygnema synadelphum* Skuja and *Zygnema coeruleum* Czurda on account of the scalariform conjugation, formation of zygospores in the conjugation-canal, and spherical zygospores with a blue scrobiculated mesospore. It further agrees with the latter species in the breadth of the cell. But, it differs from both these plants in the possession of bigger zygospores with a thick lamellated mesospore. It further contrasts with the former species in having broader cells.

The alga can also be compared with *Zygnema sinense* Jao (Jao, Studies on the Freshwater Algae of China, I, Zygnemataceae from Szechwan, Sinensia, vol. 6, No. 5, 1935) on account of the scalariform conjugation, formation of zygospores in the conjugation-canal, and sub-globose zygospores with a scrobiculated mesospore; but, it differs from the same in the possession of slightly bigger zygospores with a thick, lamellated and blue mesospore.

3. **Zygnema chalybdospermum Hansgirg** Czurda, *op. cit.*, 1932, p. 128, Fig. 133.

Forma *inflata* form. nov. (Fig. 1, C & D).

Conjugation scalariform and lateral; zygospore formation in one of the gametangia; fructifying cells swollen. Zygospores spherical to ellipsoidal; exospore thin, smooth and hyaline; mesospore thick, smooth and blue.

Lat. cell., 23. 1-26. 8 μ; long. cell., 39. 6-53 μ; crass. zygosp. spheric., 34-36.3 μ; long. zygosp. sub-spheric., 39. 6-46. 5 μ; crass. cell. fructif., 36. 3-37 μ.

Habitat:—Gorakhpur. (November 15, 1937)

The form is characterised by swollen fructifying cells and bigger zygospores.
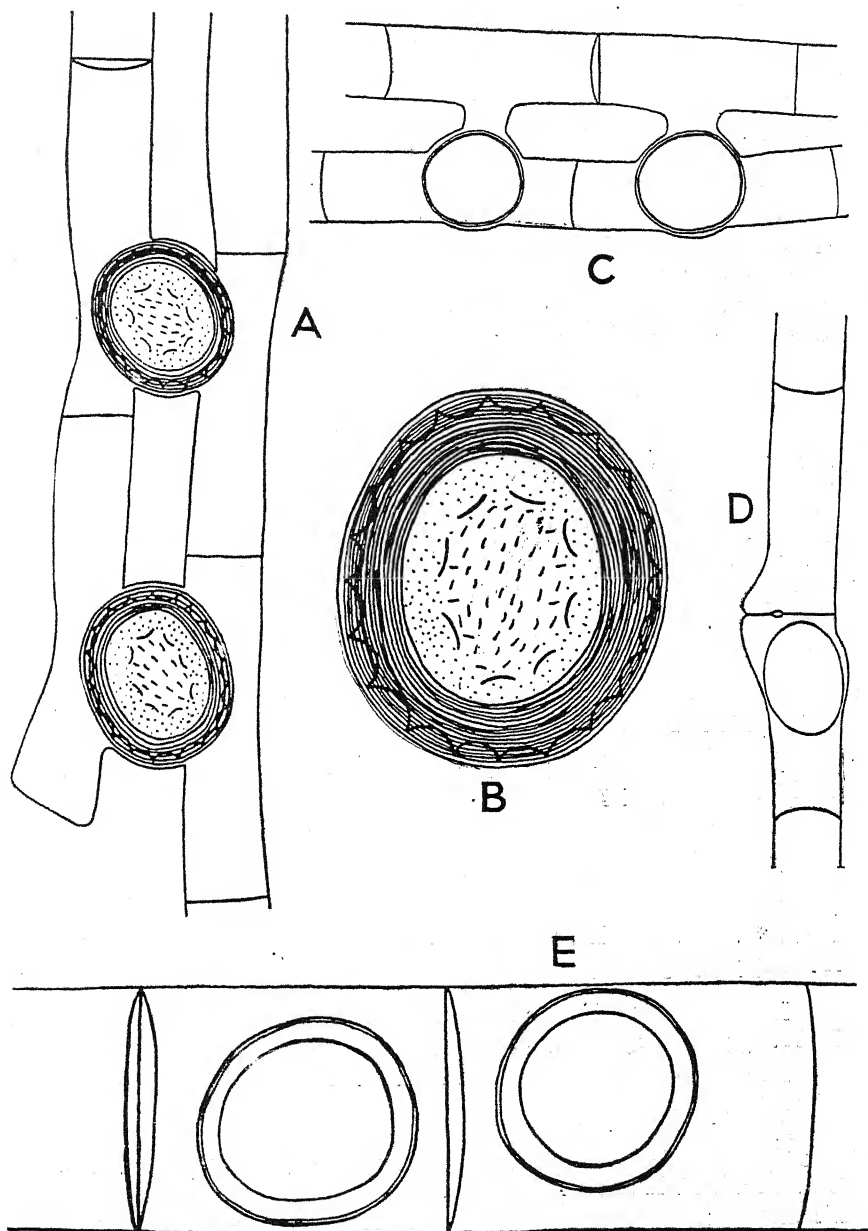
Fig. 1. A—Conjugating filaments with zygospores and B—a highly magnified zygospore showing the surface sculpture of *Zygnema gorakhporense* sp. nov.; C & D—conjugating filaments with zygospores of *Zygnema chalybdospermum* Hansgirg forma *inflata* form. nov.; E—portion of filament of *Spirogyra azygospora* sp. nov. showing azygospores. A × 416; B × 704; C & D × 180; E × 180.

Genus *Spirogyra* Link.

4. *Spirogyra azygospora* sp. nov. (Fig. 1, E).

Vegetative cells cylindrical, thrice as long as broad; end-walls plane; chloroplasts five. Reproduction by azygospores; reproductive cells unswollen. Azygospores sub-spherical to spherical; exospore thin, smooth and hyaline; mesospore thick, smooth and brown; endospore indistinct.

Lat. cell., 85-90 μ; long. cell., 270-300 μ; crass. zygosp. spheric., 60-67. 5 μ; long. zygosp. sub-spheric., 71. 25-76. 8 μ.

Habitat:—Along with *Spirogyra communis* (Hassall) Kütz. var. *intorta* var. nov., *Spirogyra nitida* Forma, Gorakhpur. (October 20, 1936; October 9, 1937).

According to the key given by Czurda (Czurda, *op. cit.*, 1932, p. 131), the alga belongs to the Section 'Mirabiles' on account of the absence of zygospore formation and the presence of azygospores. It can be compared with *Spirogyra Mirabilis* (Hassall) Kütz. and *Spirogyra Oltmannsi* Huber-Pestalozzi on account of the plane end-walls and azygospore formation. It further resembles the former species in its smooth mesospore. But, it differs from both these species in having very much broader filaments, larger number of chloroplasts and much bigger spherical azygospores. It further differs from the former species in the unswollen reproductive cells.

5. *Spirogyra varians* (Kütz.) Czurda Czurda, *op. cit.*, 1932, p. 173, Fig. 177.

Lat. cell., 32.5-35 μ; long. cell., 40-90 μ; crass. zygosp., 30-35.6 μ; long. zygosp., 39-58.5 μ.

Habitat:—In a rain-water pool, Benares. (December 2, 1937)

6. *Spirogyra longata* (Vauch.) Czurda forma Rao Rao, The Zygnemoideae of the Central Provinces, India-I Journ. Ind. Bot. Soc. Vol. XVII, 1938, p. 342.

——Fig. 1, F & G.

Lat. cell., 37.5-42.9 μ; long. cell., 80-250 μ; crass. zygosp., 33-39.6 μ; long. zygosp., 39.6-65 μ.

Habitat:—Benares. (October 30, 1937)

7. *Spirogyra subsalsa* Kütz. var. *macrospora* Rao Rao, The Zygnemoideae of the Central Provinces, India-I Journ. Ind. Bot. Soc. Vol. XVII, 1938, p. 345.

——Fig. 2, D. & E.

Lat. cell., 20.5-23 μ; long. cell., 66-190 μ; crass. zygosp., 26.4-33 μ; long. zygosp., 54-60 μ; crass. cell. fructif., 30-36 μ.

Habitat:—Sakaldiha. (October 25, 1936)

C

A

B
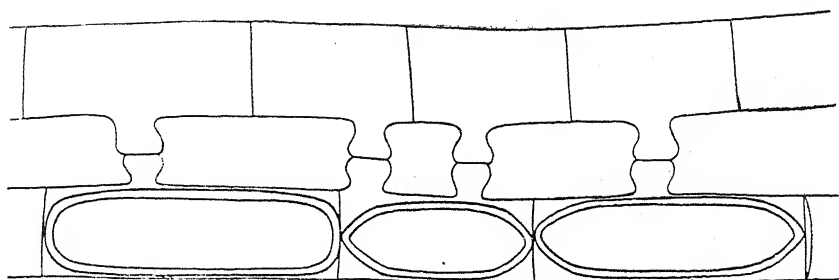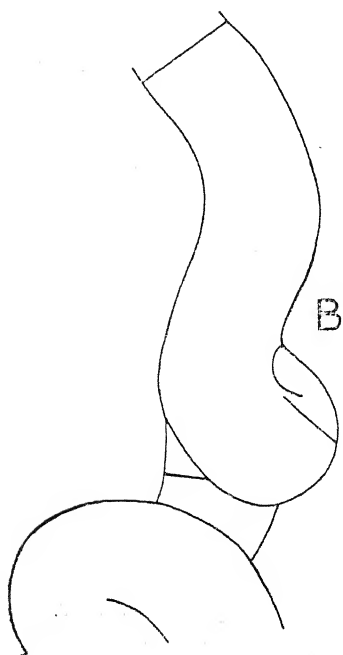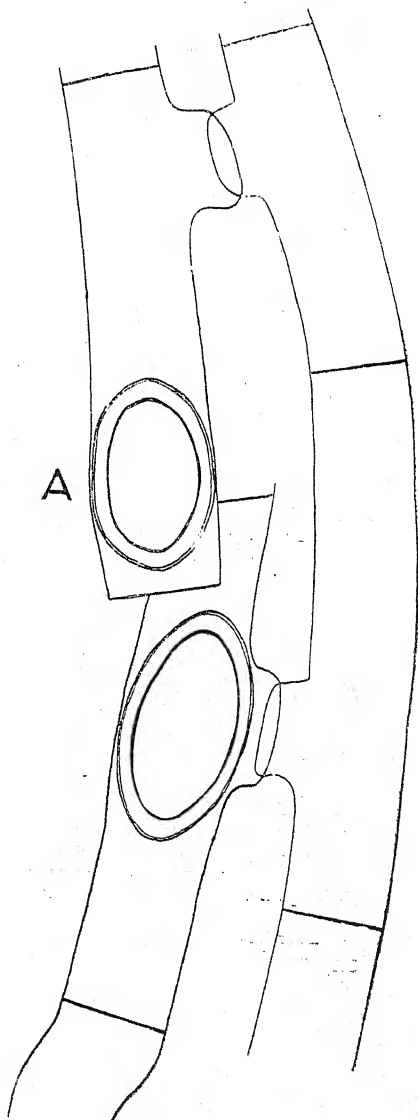
8. **Spirogyra biformis Jao** Jao, New Zygnemataceae collected in China, *American Journal* of *Botany*. vol. 23, No. 1, 1936, p. 54, Figs. 4 & 5.

Forma

Lat. cell., 45-46 μ; long. cell., 105-150 μ; crass. zygosp., 45-60 μ; long. zygosp., 90-97.5 μ.

Habitat:—Along with sterile filaments of *Spirogyra*, Gorakhpur. (October 10, 1936)

The form differs from the type in possessing slightly longer zygospores which may sometimes be a bit broader.

9. **Spirogyra Juergensii Kütz.** Borge and Pascher, in Pascher's Süsswasser-flora Deutschlands, österreichs und der schweiz, Heft 9, 1913, p. 22, Fig. 23.

Forma

Lat. cell., 30-31μ; long. cell., 112-157.5μ ; crass. zygosp., 30-34 μ; long. zygosp., 75-90 μ (41-52 μ) ; crass. cell. fructif., 31-36.5 μ.

Habitat:—Along with sterile filaments of *Oedogonium*, Gorakhpur. (November 15, 1936)

The form differs from the type in the greater thickness and variable length of the zygospores.

10. **Spirogyra communis (Hassall) K¨tz.** Czurda, *op. cit.*, 1932, p. 174.

Var. *intorta* var. nov. (Fig. 2, A & B).

Filaments usually curved or spiral sometimes straight; vegetative cells five times as long as broad; end-walls plane ; chloroplast single. Conjugation scalariform ; fructifying cells unswollen. Zygospores ellipsoidal with rounded ends; exospore thin, smooth and hyaline; mesospore thick, smooth and brown; endospore indistinct.

Lat. cell., 30–35 μ; long. cell., 115–165 μ; crass. zygosp., 26.25–30 μ; long. zygosp., 30–60 μ.

Habitat:—Along with *Spirogyra azygospora* sp. nov. and *Spirogyra nitida* Forma, Gorakhpur. (October 20, 1936)

The variety agrees with the type in the scalariform conjugation, plane end-walls, single chloroplast, unswollen fructifying cells, ellipsoidal zygospores with a thick smooth and brown mesospore; but, it differs from the same in possessing curved or spiral habit, and bigger vegetative cells and narrower spores.

11. **Spirogyra Fullebornei Schmidle** Czurda. *op. cit.,* 1932, p. 190.

Forma

Lat. cell., 43–45 μ; long. cell., 90–97.5 μ; crass. zygosp., 41–45 μ; long. zygosp., 52.5–63.3 μ; crass. zygosp. spheric., 41–43 μ.

Habitat:—Benares. (October 30, 1937)

The form differs from the type in having smaller zygospores that may in some cases be spherical.

12. **Spirogyra neglecta (Hassall) Kütz.** Czurda, *op. cit.,* 1932, p. 191, Fig. 200.

Forma

Lat. cell., about 60 μ; long. cell., 105-150 μ; crass. zygosp., 45.52·5 μ; long. zygosp., 70-85 μ.

Habitat:—Benares. (October 30, 1937)

The form differs from the type in smaller zygospores.

13. **Spirogyra szechwanensis Jao** Jao, *op. cit.,* 1936, p. 54, Fig. 6.

Var. *varians* var. nov. (Fig. 2, C).

Vegetative cells cylindrical, four to five times as long as broad; end-walls plane; chloroplasts 2-3. Conjugation scalariform; fructifying cells unswollen. Zygospores cylindric-ellipsoid to cylindrio-obovoid; exospore thin, smooth and hyaline; mesospore thick, smooth and yellow; endospore indistinct.

Lat. cell., 60-67·5 μ; long. cell., 150-285 μ; crass. zygosp., 60-65 μ; long. zygosp., 135-180 μ.

Habitat:—Along with some sterile filaments of *Spirogyra* and *Oedogonium*, Gorakhpur. (October 15, 1936)

The variety agrees with the type in the plane end-walls, scalariform conjugation, cylindric-ellipsoid zygospores with a thin smooth and hyaline exospore and a smooth and yellow mesospore; but, it differs from the Chinese form in the narrower cells, in the usually smaller number of chloroplasts and in the zygospores being commonly cylindric-obovoid completely filling the fructifying cells.

7
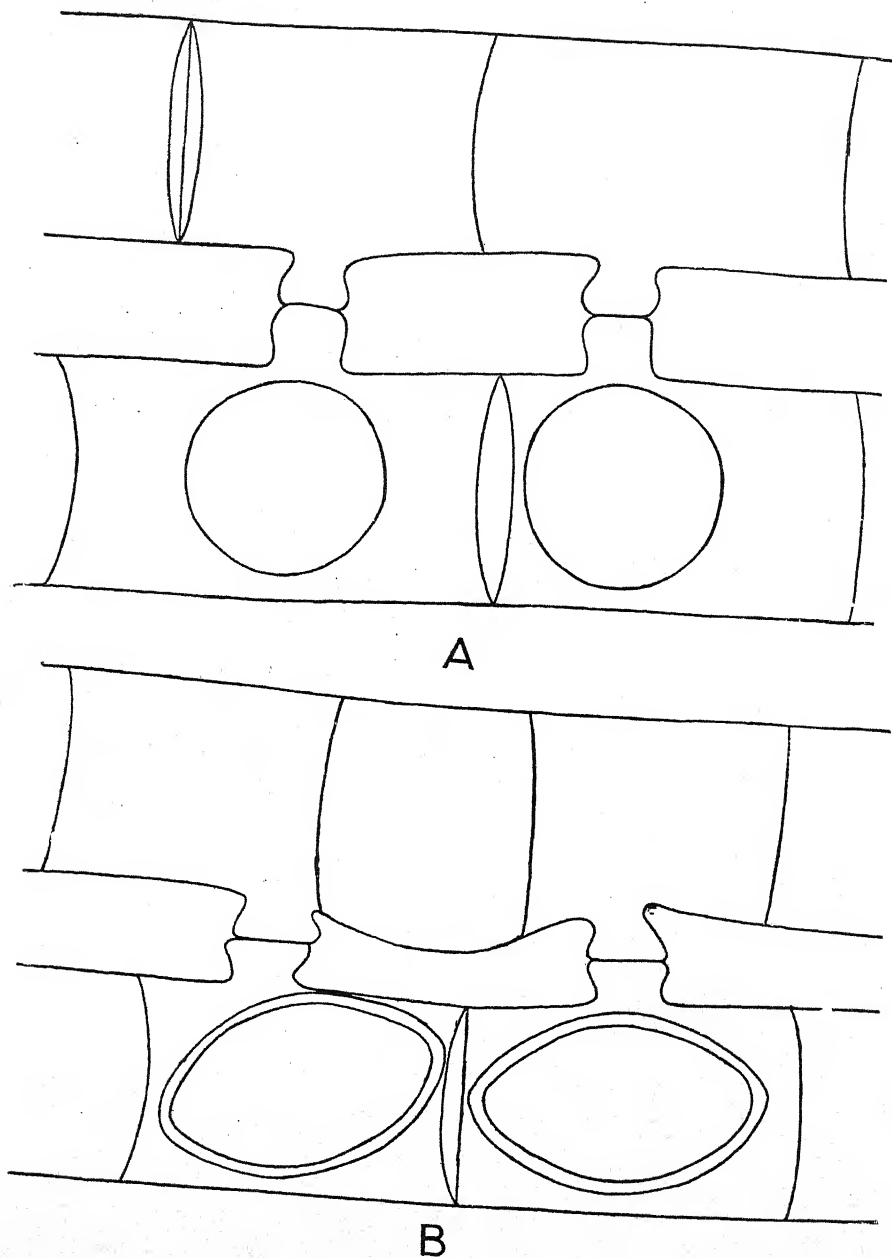
Fig. 3. A—Conjugating filaments with zygospores of *Spirogyra margaritata*
Wollny. var. *microspora* var. nov., B—conjugating filaments with zygospores of
Spirogyra ellipsospora Transeau forma *tenuis* form. nov. A×180; B×184,

14: *Spirogyra nitida* (**Dillwyn**) **Link.** Borge and Pascher, *op. cit.*, 1913, p. 26, Fig. 37; Jao, *op. cit.*, 1935, Pl. VI. Figs. 70 & 71.

Forma

Lat. cell., 75 μ; long. cell., 225-270 μ; crass. zygosp., 75 μ; long. zygosp., 120-135 μ.

Habitat : —Gorakhpur. (November 15, 1936)

The form differs from the type in the occasional presence of longer cells.

15. *Spirogyra margaritata* **Wollny.** Czurda, *op. cit.*, 1932, p. 192, Fig. 201.

Var. *microspora* var. nov. (Fig. 3, A.)

Vegetative cells cylindrical, four times as long as broad; end-walls plane; chloroplasts 7-8. Conjugation scalariform; spore-wall indistinct.

Lat. cell., 75-90 μ; long. cell., 120-300 μ; diam, zygosp., 60-67·5 μ.

Habitat :—Gorakhpur, (November 15, 1936)

The variety agrees with the type in the plane end-walls, scalariform conjugation, unswollen fructifying cells, spherical zygospores with indistinct spore-walls; but, it differs from the same in possessing narrower cells fewer chloroplasts and smaller zygospores.

16. *Spirogyra ellipsospora* **Transeau** Czurda, *op. cit.*, 1932, p. 202, Fig. 217.

Forma *tenuis* form. nov. (Fig. 3, B.)

Vegetative cells cylindrical; end-walls plane; chloroplasts 6-8. Conjugation scalariform; fructifying cells unswollen. Zygospores ellipsoidal; exospore thin, smooth and hyaline; mesospore thick, smooth and brown; endospore indistinct.

Lat. cell., 120-135 μ; long. cell., 150-180 μ; crass. zygosp., 105-135 μ; long. zygosp., 165-195 μ; crass. conjugation-canal., 45-55 μ.

Habitat :—Gorakhpur. (November 20, 1937)

The form possesses slightly drawn-out ends of the zygospores and narrower conjugation-canals.

17. **Spirogyra plena (W. et G. S. West) Czurda** Czurda,
op. cit., 1932, p. 193, Fig. 203.

Forma

Lat. cell., 42.9-46.2 μ ; long. cell., 80-90 μ ; crass. zygosp.,
39.6-42.9 μ ; long. zygosp., 62.7-70 μ.

Habitat :—Gorakhpur. (November 15, 1936)

The form differs from the type in possessing bullate sterile
cells in the conjugating filaments and broader zygospores.

18. **Spirogyra crenulata sp. nov.** (Fig. 4, A & B).

Vegetative cells cylindrical, seven to eight times as long as
broad ; end-walls plane ; cloroplasts two. Conjugation scalari-
form , conjugation tubes formed wholly by the male gametangia ;
fructifying cells swollen, nearly forming quadrangles. Zygo-
spores obovoid ; spore-wall of five layers ; outer exospore thin
and hyaline ; inner exospore colourless, more or less lamellose,
and upto 3.5 μ thick ; outer mesospore thin, yellow and a little
wrinkled ; inner mesospore yellowish-brown and reticulate, reti-
culations thick and irregularly crenulate to dentate ; endospore
thin and distinct.

Lat. cell., 29.6-36.3 μ ; long. cell., 158.4-330 μ ; crass.
zygosp., 52.8-60 μ ; long. zygosp., 67.5-93.3 μ ; crass. cell.
fructif., 56.1-61 μ.

Habitat :—Gorakhpur. (October 15, 1936)

The alga belongs to the Section ' Conjugata ' and can be
compared with *Spirogyra aequinoctialis* G. S. West and *Spirogyra
Schmidtii* W. & G. S. West on account of the two *chloroplasts*,
scalariform conjugation and swollen fructifying cells. It further
agrees with the latter species in the breadth of the filament.
But, it differs from both the species in the obovoid zygospores
with five-layered spore-wall, outer exospore thin and hyaline,
inner exospore colourless, more or less lamellose, and upto 3.5 μ
thick, outer mesospore thin, yellow and a little wrinkled, inner
mesospore yellowish-brown and reticulate, reticulations thick and
irregularly crenulate to dentate, endospore thin and distinct. It
further differs from both of these species in the formation of
conjugation-canal wholly by the male gametangia, it also
contrasts with the former species in the broader and larger
zygospores and from the latter in the broader and smaller
zygospores.

The alga can also be compared with *Spirogyra corrugata*
Transeau (Transeau, Tiffany, Taft and Li, New Species of
Zygnemataceae, *Trans. Micro. Soc.*, vol. LIII, No. 3, 1934, Pl.
XXI, Fig. 63.) on account of the plane end-walls, scalariform
conjugation, conjugation-canal formed wholly by the male
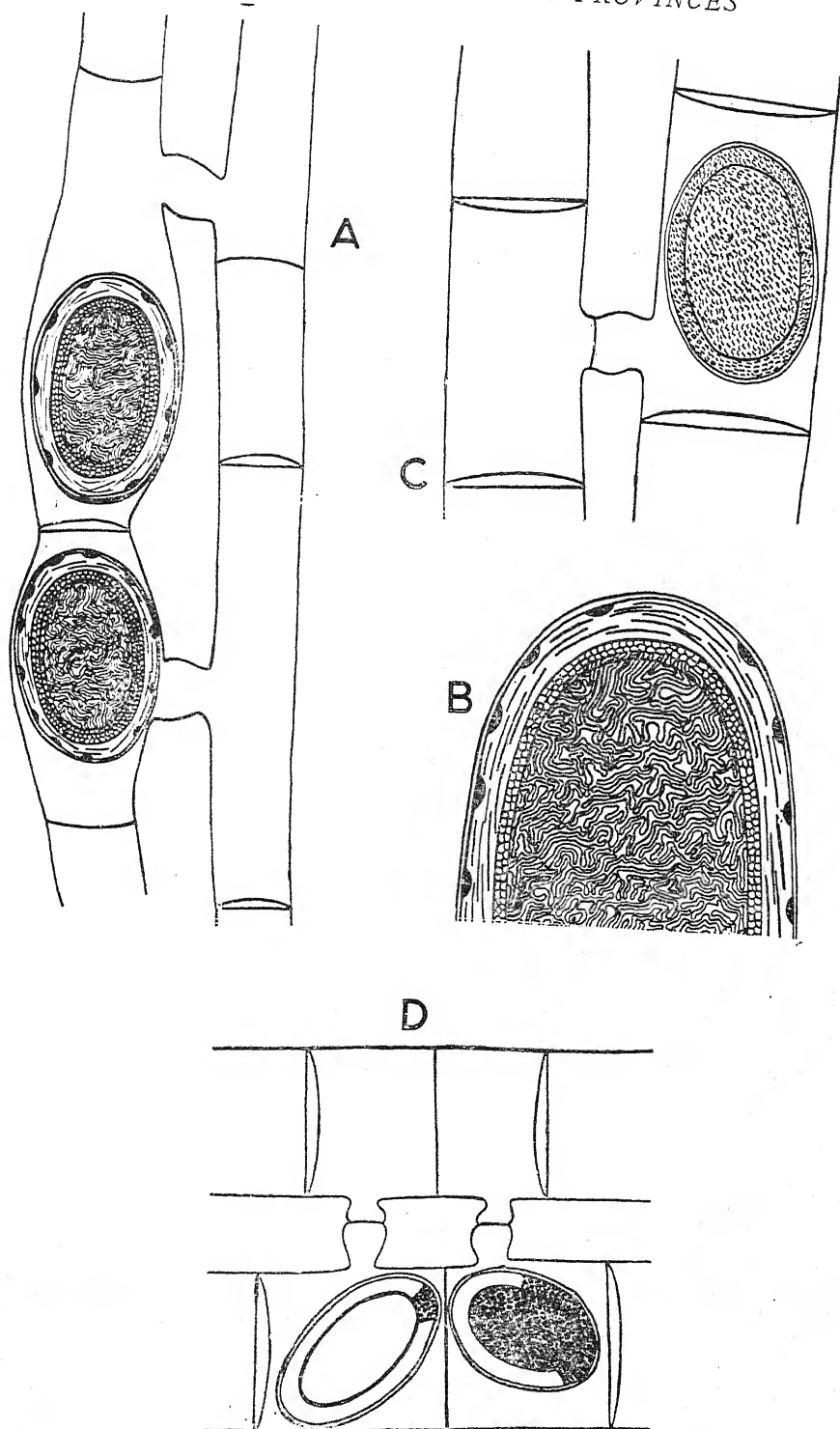
Fig. 4. A—Conjugating filaments with zygospores and B—a portion of which

gametangia, swollen fructifying cells, obovoid zygospores with yellow mesospore; but, it differs from the same in the narrower and longer cells, in smaller zygospores and in the nature of the spore–wall.

19. **Spirogyra verruculosa Jao** Jao, *op. cit.*, 1936, p. 59 Figs. 32 & 33.

Var. *crassa* var. nov. (Fig. 4, C.)

Vegetative cells twice as long as broad; end–walls plane; chloroplasts five. Conjugation scalariform; fructifying cells unswollen. Zygospores ellipsoidal with rounded ends; exospore thin, smooth and hyaline; mesospore thick, yellowish–brown and verrucose; endospore indistinct.

Lat. cell., 90–105 μ; long. cell., 195–210 μ; crass. zygosp., 71.3–78.8 μ; long. zygosp., 120–131.3 μ.

Habitat :—Gorakhpur. (October 15, 1936)

The variety agrees with the type in the scalariform conjugation, unswollen fructifying cells and ellipsoidal zygospores with a thin smooth and hyaline exospore and thick yellowish-brown and verrucose mesospore; but, it differs from the same in having narrower cells and smaller zygospores.

It also agrees with *Spirogyra verruculosa* Jao, var. *chakiaense* Rao, but differs from the same in possessing broader cells and larger zygospores with rounded ends.

20. **Spirogyra anamola Rao.** Rao, *op. cit.*, 1937, p. 284, Fig. D & E.

Forma (Fig. 4, D.)

Lat. cell., 97.5–101.3 μ; long. cell., 90–105 μ; crass. zygosp., 75–76 μ; long. zygosp., 97.5–106.5 μ; crass. cell. fructif., 105 μ.

Habitat :—Gorakhpur. (October 20, 1936)

The form differs from the type in having narrower cells and shorter zygospores with rounded ends. It further differs in possessing lesser number of chloroplasts and in sometimes slightly swollen fructifying cells.

21. **Spirogyra kundaensis sp. nov.** (Fig. 5. A & B).

Vegetative cells cylindrical, as long as broad or longer than broad; end-walls plane; chloroplasts 4-5. Conjugation scalariform; fructifying cells unswollen. Zygospores ellipsoidal with rounded ends; exospore thin, smooth and hyaline; mesospore
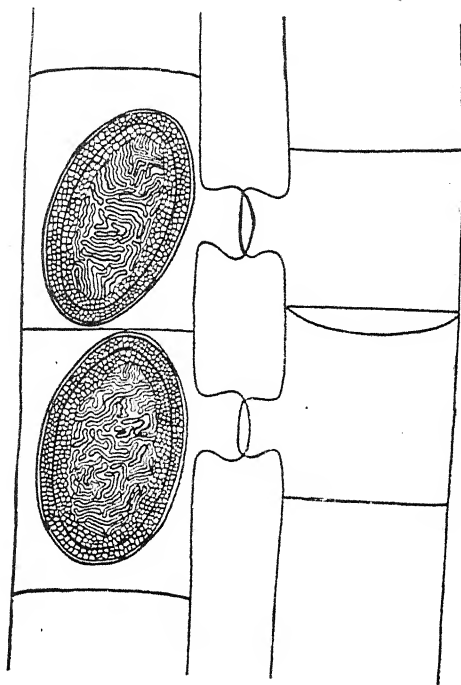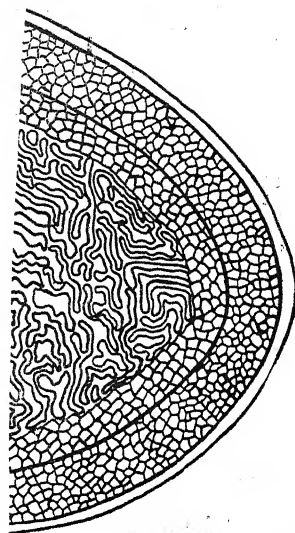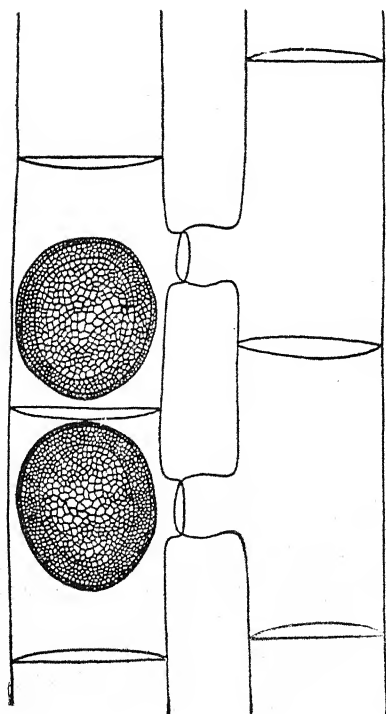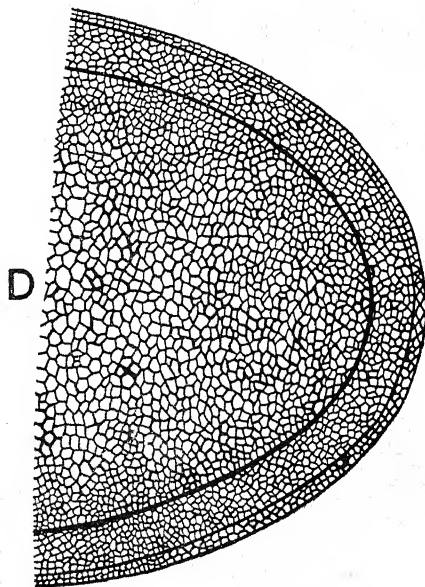
thick, brown and reticulate, reticulations thick and irregularly crenulate to dentate; endospore indistinct.

Lat. cell., 105-120 ; long. cell., 150-180 ; crass. zygosp., 75-90; long. zygosp., 105-135 μ.

Habitat :—Kunda Ghat, Gorakhpore. (October 19, 1936).

The alga belongs to the Section 'Conjugata' of the genus and may be compared with *Spirogyra Reinhardii* Chmielewski and *Spirogyra paraguayensis* Borge on account of the scalariform conjugation and ellipsoidal zygospores with sculptured mesospore. It further agrees with the former species in the number of chloroplasts and thickness of the filaments, and with the latter species in the unswollen fructifying cells. But, it differs from both in the sculpture on the mesospore being reticulate, reticulations thick and irregularly crenulate to dentate. It further differs from the former species in the unswollen fructifying cells and narrower zygospores that are frequently smaller, and with the latter species in having much broader filaments and bigger zygospores.

The alga can also be compared with *Spirogyra anamola* Rao in scalariform conjugation, ellipsoidal zygospores with a sculptured mesospore ; but, it differs from the same in the lesser number of chloroplasts, zygospores with rounded ends and in the sculpture on the mesospore being reticulate, reticulations thick and irregularly crenulate to dentate.

22. *Spirogyra Ghosei* sp. nov. (Fig. 5, C. & D).

Vegetative cells cylindrical, longer than broad ; end-walls plane ; chloroplasts 6-7. Conjugation scalariform ; fructifying cells unswollen. Zygospores ovoid with rounded ends ; exospore thin, reticulate ; mesospore thick and brown, and reticulate; both showing a close net-work ; reticulations thick and regular; endospore distinct.

Lat. cell., 100-105 μ; long. cell., 225-390 μ; crass. zygosp., 90-102.5 μ; long. zygosp., 105-120 μ; crass. conjugation-canal, 45-50 μ.

Habitat:—Along with sterile filaments of *Oedogonium*, Gorakhpur. (November 8, 1937)

The alga may be compared with *Spirogyra anamola* Rao on account of the scalariform conjugation, plane end-walls and reticulate mesospores but, it differs from the same in ovoid zygospores with rounded ends, and in both exospore and mesospore showing a close net-work. It further differs in smaller number of chloroplasts and broader zygospores.

Fig. 6. A-Conjugating filaments with zygospore of *Sirogonium ventersicum* Transeau forma *variabilis* form. nov. ; B-the same of *Sirogonium indicum* sp. nov A & B×462.
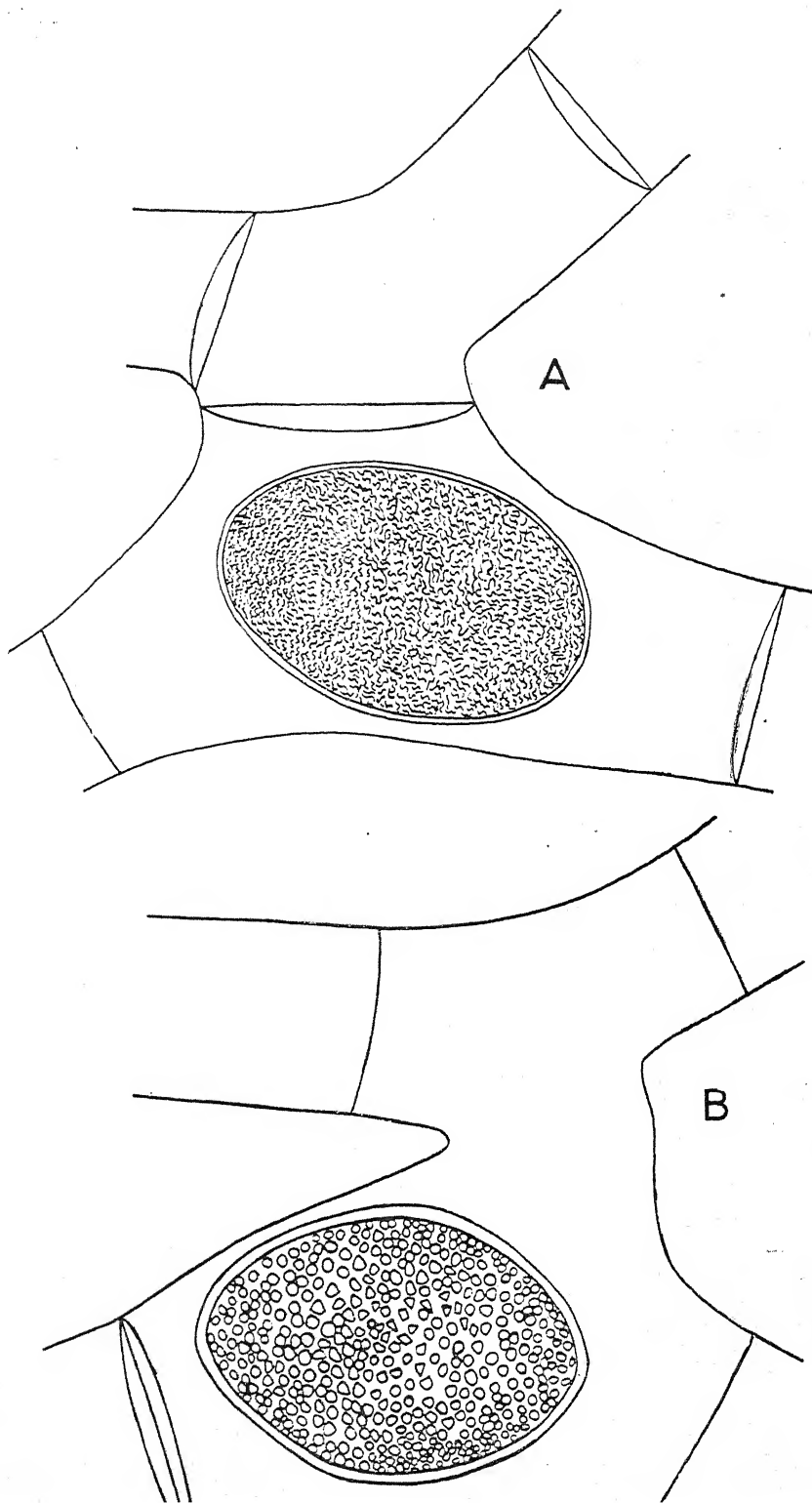
(b) **Mougeotiaceae.**

Genus *Mougeotia* Agardh.

23. *Mougeotia recurva* (**Hassall**) **de Toni** Czurda, *op. cit.*, 1932, p. 67, Fig. 41.

Lat. cell., 13.3–15 μ; long. cell., 112.5–200 μ; crass. zygosp. spheric., 25–26.5 μ.

Habitat :– Gorakhpur (October 25, 1937).

Genus *Sirogonium* Kütz.

24. *Sirogonium ventersicum* **Transeau** Transeau, Tiffany, Taft and Li, *op. cit.*, 1934, Pl. XXII, Fig. 65.

forma *variabilis* form. nov. (Fig. 6, A).

Vegetative cells upto four times as long as broad ; end-walls plane ; chloroplasts 7-8, straight or making one turn : fructifying cells inflated. Zygospores ellipsoidal to kidney–shaped to subspherical ; exospore thin, smooth and hyaline ; mesospore thin, brown, densely and irregularly verrucose.

Lat. cell., 60–75 μ; long cell., 210–240 μ; crass. zygosp., 60–90 μ ; long. zygosp., 135–195 μ ; crass. cell. fructif., 100-150 μ.

Habitat:— Gorakhpur. (October 15, 1937)

The form is characterised by variable shapes of zygospores which are sometimes very much bigger.

25. *Sirogonium indicum* sp. nov. (Fig. 6, B)

Vegetative cells upto four times as long as broad ; end-walls plane; chloroplasts 7, straight; fructifying cells inflated. Zygospores ellipsoidal with rounded ends; exospore thin, smooth and hyaline; mesospore thick, yellow and irregularly scrobiculate.

Lat. cell, 60-80 μ; long. cell., 210-285 μ; crass. zygosp., 75-90 μ; long. zygosp., 135-165 μ; crass. cell. fructif., 120-125 μ.

Habitat :—Gorakhpur. (November 2, 1937)

The alga belongs to the Section 'Sirogonium' and can be compared with *Spirogyra ceylanica* Wittrock and Nordstedt on account of the plane end-walls, number of chloroplasts, knee-shaped conjugation and ellipsoidal zygospores. It, however, differs from the same in the smaller zygospores with an yellow and irregularly scrobiculate, mesospore scrobiculations being smaller and irregular.

In conclusion, I have great pleasure in expressing my great indebtedness to Professor Y. Bhâradwâja, for his kind guidance and criticism throughout the course of this investigation.

REVIEW

Bulletin of the Madras Government Museum. Supplement to the Flowering Plants of Madras City and its immediate neighbourhood by E. Barnes, B. Sc., Madras Christian College, Tambaram; Edited by the Superintendent. *New Series*—Natural History Section, Vol. IV. No. 2, Government Press, Madras, 1938. Price Re. 1-10-0.

In 1929, the flora on which this supplement is based was published rather in haste before a thorough survey of the small area dealt with had been made, as the author of this supplement remarks in his preface ; the only justification for such action being that Gamble's flora of the Presidency of Madras had not been completed at the time (1929). This Supplement even is not a complete list of all not included in the flora previously published, as it excludes grasses and other plants.

Sir D. Prain in his preface to the Bengal Plants, referring to the completion of the Flora of British India, justly remarks that one period having ended a new one must begin. The attention of systematists must now be directed to the compilation of smaller works, compact in form and concise in style, dealing with the vegetation of specific areas within that Indian Empire which is served by the Flora of British India. So it is befitting that endeavour of local botanists be directed towards making up small lists of plants growing on specific areas. But it is desirable that such lists should be carefully made inclusive of all plants growing in particular localities, so that they might not require to be supplemented again and again in the course of a very few years.

This supplement contains some 50 species with their elaborate specific description, key, etc., covering 46 quarto pages making the whole unnecessarily voluminous. Of course, the list of plants appended at the end is a welcome addition.

Some of the species in the present list, such as, *Pterocarpus Marsupium, Mimosa rubicaulis, Anogeissus latifolia, Randia uliginosa, Gnaphalium indicum, Lobelia trigona, Trichodesma zeylanicum, etc.*, are not confined to Madras and its neighbourhood alone, but extend far outside the limits of the Presidency and are seen in Orissa, Bihar and other places.
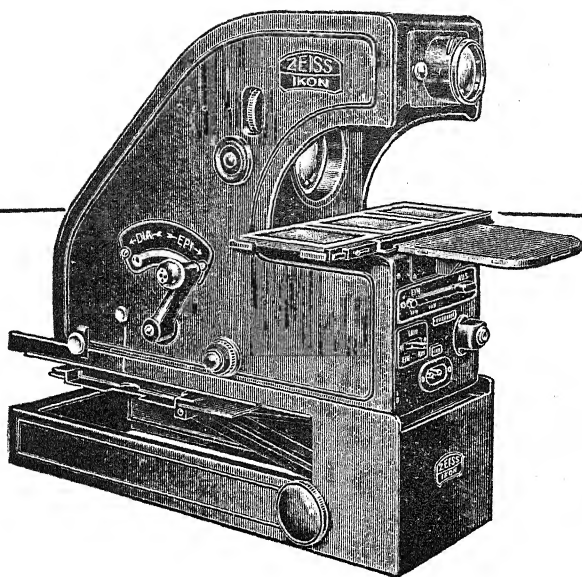
The tentative keys to the species are not always happy ones, for instance, for *Mimosa rubicaulis,* the key runs thus : " differs from the 2 species of *Mimosa* as follows from *M. pudica* (L) in being a large bush from *M. hamata* (Willd) in having leaves upto 6 ins. long." These are all very variable vegetative characters and no conclusive distinction can be drawn from such vague points. In Prain's Bengal Plants the following key is given for

M. rubicaulis (Lamp)—"Stamens twice as petals, rachis of leaf smooth, stem prickly but not bristly."

The phenological notes given after the description of the species are from personal observation and therefore are of value, but in some cases their importance suffers and is of local value only because of observation being limited to a particular locality.

<div align="right">D. M.</div>

CHRONICA BOTANICA

P. O. Box 8

LEIDEN – HOLLAND

From February 1938 **Chronica Botanica** will be issued **bi-monthly** and no longer as a year-book. The annual subscription will be reduced from 15 to 7 guilders. The new periodical will continue to give all the essential information which was given in the old year-book and will include some important new sections as well. Like the year-book, the new Chronica will **aim at promoting documentation, goodwill and intern. cooperation among plant scientists.** Results of research will be published only in the first two sections. The world list of plant science institutions and societies will appear as an annual supplement. The contents of the reorganized Chronica will be as follows:
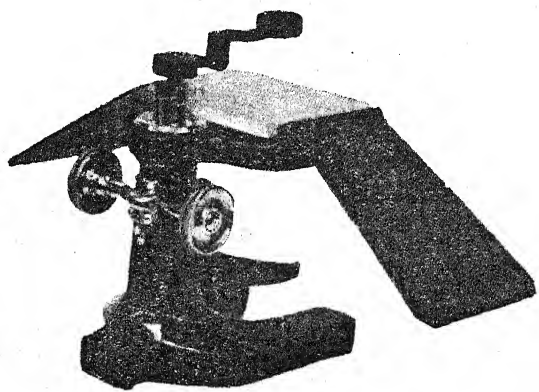
1. **Scientific Communications:** a medium for the quick publication of short preliminary notes on the results of recent research or announcing new discoveries.

2. **Forum Botanicorum:** Discussions, Announcements, Letters to the Editor.

3. **Intern. Congresses:** Detailed programmes, short reports, decisions, resolutions, etc.

4. **Quotations:** from recent articles of general and timely interest.

5. **Miscellaneous News:** News notes of all kinds of plant science institutions, experiment stations, gardens, etc., incl. notes on new research projects.

6. **Herbarium and Museum News:** Expeditions, new collections, lists of new acquisitions, etc.

7. **Personalia:** Appointments, Retirements, Resignations, Deaths (short obituaries), Miscellaneous, New Addresses.

8. **Queries:** Requests for cooperation and information, exchange offers.

9. **New Periodicals:** Short accounts of new plant science periodicals, changes in existing periodicals.

10. **New Books:** Short reviews of new plant science books.

NOTE TO CONTRIBUTORS

Only papers written or **communicated by** Members of the Indian Botanical Society are published in the Journal.

Attention to the following points will greatly assist the Editor and ensure early and satisfactory publication :—

Manuscripts must be typed with double spacing and on one side of the paper only. Authors are particularly requested to revise their manuscripts carefully before submitting them to the Editor. This will reduce the corrections in the proof.

In view of the high cost of publication, contributions should be as concise as possible and all unnecessary tables and illustrations should be avoided. If the contributions are very long, the authors may be required to contribute a portion of the cost of publication.

Names of genera and species should be underlined and will appear in italics. The names of the authors of genera and species should always be given.

Original papers must conclude with a summary, drawing attention to the main facts and conclusions. References to literature cited should, as far as possible, be complete and must be carefully verified. A bibliography should be given at the end of the paper arranged alphabetically under authors' names.

References to literature in the text should be made by quoting the author's name and the year of publication adding the page where possible, thus (A. B. 1934, p. 25). When the author's name occurs as a part of the text, only the year and page need be given. No references should be given as footnotes.

Illustrations in line.—These should be drawn boldly in Indian ink on Bristol board or smooth white card. All necessary shading must be done in well-defined dots or lines. Colour, either in line or wash, should be avoided. It is important that such illustrations be drawn at least twice as large (in linear dimensions) as they are to appear in the reproduction. Due allowance for reduction must be made in size of lettering, thickness of line, and closeness of shading. Where possible the figures should be grouped so as either to fit the page after reduction, or to come on part of the printed page as text figures. Authors should number the text-figures consecutively and indicate in the text the places where they are to appear.

Wash Drawing.—For making half-tone blocks the original drawings should be done with brush and not with pen and ink. For these either black or gray colour may be used. Black is better than gray.

Photographs for block-making.—Every photographic print from which blocks are required should be black and white on glossy surfaced paper—if possible extra glazed. The prints must be sufficiently washed at the time of making them, since insufficiently washed prints become yellowish in colour and fade soon afterwards. Though every photograph will reproduce as a half-tone block, best results are obtained only from black and white prints on glossy paper. Photographic prints on matty paper, sepia-toned prints or faded prints will not give clear results in block-making. The numbering or lettering inside the photographs should be done in pen only either with undiluted Indian ink on white surface or with Chinese white on black surface. In case the photographs are to be reduced for reproduction the numbers or letters should be drawn large enough to allow for the necessary reduction Combination of photographs and pen drawings in one plate should be avoided.

Allowance of space should be made for legends below the illustrations.

Graphs should be drawn in Indian ink on co-ordinate paper ruled with blue lines Any co-ordinate which is desired to appear in the reproduction should be drawn over with Indian ink.

Drawings or photographs grouped for half-tone production should be trimmed nd fitted together perfectly so that they completely fill the board on which they are mounted. The cut edges always show in the half-tone reproduction while they do not appear in zinc-etchings.

Authors should correct the galley proofs and should make only the necessar changes. Extensive changes mean delay and extra cost which latter is chargeable to the authors.

Each author will receive *gratis* 50 copies of the printed paper. Extra copies will be supplied at Printer's cost price; the number of extra copies required must be stated on the corrected proof.

Reviews or abstracts of books or of papers in other Journals should begin with the author's name and initials, followed by the title of the book or paper, the name volume, number and exact pages of the serial where the paper was published, the publisher's name, the place and date of publication and preferably, in the case of books, the price.

All matter intended for publication in the Journal should be sent to the Chief Editor, P. Parija, M.A. (Cantab), Professor of Botany, Ravenshaw College, Chauliagunj P.O., Cuttack.